LABORATORY DIRECTIONS

IN

GENERAL BIOLOGY

First Edition.

BY

EDWIN G. CONKLIN,

Professor of Biology

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PRESS

THE AIMS AND METHODS OF LABORATORY WORK IN BIOLOGY.

The purpose of all laboratory work is to study nature at first hand. Its educational value lies chiefly in the cultivation of accuracy and independence both of observation and of judgment. Each student is expected to make for himself the observations and experiments hereafter indicated.

A record of every observation or experiment must be entered in the prescribed note book, under numbers corresponding to those in these Directions. This record should consist of drawings and descriptive notes, and every page should bear the name of its author and the date. The record for each topic must be inspected and passed by an instructor before any new topic may be undertaken.

To each student in the laboratory is assigned a locker containing a microscope, reagents, glassware, etc., for the safe keeping of which he is held responsible. The microscope is the most complex and delicate instrument in this outfit and work with it should be preceded by a study of the following description of its parts and directions as to its use.

I. THE MICROSCOPE.

A.—DESCRIPTION. The body or *tube* bears the lenses and is supported upon a *stand* which also carries a *mirror* to cast light upon the object examined through a hole in the flat *stage* upon which the object is placed. This hole can be made of various diameters by means of *diaphragms*. A lens for concentrating light, and known as a *condenser* may be placed between the mirror and the stage. The lens at the upper end of the tube is the *ocular* or eye-piece; there are two oculars, of different magnifying power. The combination of lenses at the lower end of the tube is the *objective;* in this microscope there are two objectives of different magnifying power, one marked 3, the other 6; the former (low power objective) is in focus, i. e., gives a clear image of the object examined when its lower end is about ⅔ in. above the object, the latter (high power objective) is in focus when it is about ⅙ in. above the object. The objectives are carried upon a *nose-piece* by revolving which either

one of them may be brought to lie at the lower end of the tube. The tube is really double, one tube being telescoped within another. By holding the body firmly in the left hand and taking hold of the projecting brass ring just below the eye-piece with the right, the inner tube may be drawn out some distance, thus lengthening the body and increasing the magnification. The length of the tube without lenses and nose-piece is 150 mm. and it can be drawn out to 195 mm.; with the nose-piece the tube is 10 mm. longer. A table of magnifications of the different lenses with a given tube length is found on the inside of each case.

B.—USE. 1. Reflect light, from white clouds if possible, upon the object. Where all the light is needed, use *concave mirror;* where light is intense and a low magnification is required, use *plane mirror.*

2. Use smaller diaphragms with higher powers.

3. To focus lenses upon an object, use first the *coarse*, then the *fine adjustment;* the former movement is by means of a *rack and pinion.* The rack is the toothed plate along the back of the tube, the pinion is a small cog wheel which fits into the rack and is turned by the two *milled wheels* on each side of the tube. The *fine adjustment* is by means of a *milled screw* back of the pinion. If the fine adjustment screw is turned in the direction in which a clock's hands move the tube is lowered, turned in the reverse direction it is raised. In using a high power turn the tube down nearly to the object and, then, while looking through the microscope, bring the object into focus by slowly turning the tube upward. Never focus *down* upon the object, since by this method there is danger of crushing the lens into the object. Keep one hand on the fine adjustment when looking at an object and vary the focus constantly to bring all the fine details of structure into view.

4. Do not use higher power objective without *cover glass* over object examined.

5. Always use the lower power before the higher one; and always use the lowest possible power sufficient for distinct vision.

6. Do not touch lenses with fingers. If the field is blurred or the object dim either the cover glass or the lenses are at fault. If the cover glass is dirty remove it and clean it; if the fault is in the eye-piece the particles of dirt revolve when the eye-piece is rotated. If the field is still dim the objective is dirty and must be removed and cleaned. In cleaning the lenses never use anything but clean tissue paper supplied for that purpose. If necessary, the

lenses may be moistened by breathing upon them; if this is not sufficient, consult the instructor.

7. Keep both eyes open, using either the right or the left. The strain of microscopic work on the eyes is usually due to the fatigue of constantly closing one eye. If you cannot see the object with both eyes open use the eye-shade provided for that purpose.

8. Never leave the laboratory without first placing the microscope in its case and locking it and all your apparatus in your locker.

II. PREPARING OBJECTS.

The preparation of objects for examination under the microscope is termed *mounting*. Objects are usually mounted on pieces of glass 3 x 1 in., known as *slides*. Observe the following directions:

1. If the object to be studied is a mass of cells, separate it into very small pieces by means of *teasing needles;* if it is a fluid use only a very small drop. If too much fluid has been used it will run out from under the cover glass and the excess must then be soaked up with filter paper. Temporary preparations are usually mounted in water, permanent ones in balsam.

2. The lenses of the microscope, the upper side of the cover glass and the lower surface of the slide must be perfectly clean and dry.

3. Having placed the object in a small drop of mounting fluid take a cover glass in your left hand, rest one edge of the cover on the slide near the drop and support the opposite edge on a teasing needle; lower the cover glass gradually over the drop, being careful to inclose no air bubbles. Do not press upon the cover glass.

4. Before putting a permanent preparation away label it carefully with the name of the object and the method of preparation.

5. Never use reagents hap-hazard but only when you have a definite purpose in view. Reagents are used for fixing, hardening, preserving, staining, dehydrating, clearing, embedding and mounting. *Fixing* is the process of killing and hardening the living thing so that it preserves as nearly as possible its natural form. *Staining* is the dyeing of the object so that some parts are more deeply colored than others. *Dehydrating* is the process of removing the water from the object, usually by alcohol. *Clearing* usually consists in substituting some oil for the alcohol which is in the object. *Embedding* is the process of permeating and surrounding the object with some substance such as paraffin, preparatory to cutting sections of it.

III. NOTES AND DRAWINGS.

1. Drawings should be made of every object studied; this is necessary not only as a record of what has been seen, but also as an aid to accurate observation. In general make outline drawings only. Use hard pencils (4H-6H), with very sharp points, and make the drawings large enough so that all details can be represented without confusion. Where certain structures occur in large numbers it is sufficient to represent them in only a part of the drawing. Label all important structures by means of reference lines and marginal words.

2. To draw to scale:—Place paper at base of microscope and endeavor to trace outlines as seen with left eye while seeing point of pencil at same time with right eye. The pencil point must appear to coincide with the part of the object being drawn. Do not move the eyes.

3. To make a scale:—Focus upon the lines drawn one-tenth and one-hundredth of a millimeter apart upon a stage micrometer. With a camera lucida draw several of the lines upon a card. Make such a scale for each combination of lenses. Carefully label the card with the lenses used and lay it away for future use.

IV. EXAMINATION OF COMMON OBJECTS.

1. Put a few fibres of wool, cotton, linen and silk in a drop of water on a slide, cover with cover glass and examine first under a low power, then under a high one. How do the fibres differ? Sketch one of each and label them.

2. Examine a drop of oil emulsion (oil suspended in water) and notice peculiar effects of refraction when lenses are focused upon different portions of a drop.

3. Examine bubbles of air in water. These may be obtained by running in water under a cover glass supported at one side by a bit of paper and then tapping on the cover glass with a needle. What differences can you see between these and oil drops?

Part I

GENERAL PHYSIOLOGY AND MORPHOLOGY

A. CHEMICAL AND PHYSICAL CHARACTERISTICS OF LIVING THINGS.

The bodies of all living things are composed of about 15 chemical elements and a great number of chemical compounds: 97 per cent of the human body consists of carbon, hydrogen, oxygen and nitrogen, and 3 per cent of 11 other elements. Three-fourths of all the hydrogen and nine-tenths of all the oxygen are combined to form water. In addition to water and mineral salts living things contain carbon compounds, or "organic compounds." Compounds of carbon, hydrogen and oxygen form *Carbohydrates* or *Hydrocarbons;* compounds of carbon, hydrogen, oxygen and nitrogen form *Proteids.*

I. CARBOHYDRATES (Starches, Sugars).

Carbohydrates of physiological importance are:—

Polysaccharids $(C_6 H_{10} O_5)^n$—starch, dextrin.

Monosaccharids $(C_6 H_{12} O_6)$—dextrose.

Disaccharids $(C_{12} H_{22} O_{11})$—cane sugar.

1. a. Polysaccharids. Native starch. Mount a scraping of potato in water and examine under microscope. Study and draw structure of starch grains. Run a drop of iodine solution under cover. What happens?

b. Grind a little commercial starch in a mortar and shake with cold water. Filter and test filtrate with iodine.

c. Test solubility in boiling water. Note character of resulting solution. Dilute and add a drop or two of iodine solution. What results? Heat and cool again noting result. Add (1) alkali, (2) alcohol to the colored solution. What results?

d. Cellulose (plant cell-walls). Cotton fiber is almost pure cellulose. Note insolubility in water and alcohol. Is it insoluble in acids? Alkalies? Does it react with iodine? Treat with 20 per cent sulphuric acid and then add iodine. What results? Treat with Schultze's chlor-zinc-iodide. What results? This is known as the "cellulose test."

2. Monosaccharids (grape sugar, etc).

a. Dextrose. Make a one per cent solution in hot water. Test solution as follows:—

(1). Add iodine. Result? Is starch present?

(2). Add ¼ its volume of strong potassium hydroxide and heat gradually to boiling point. Note color and odor (Moore's test).

(3). Add a few drops of copper sulphate solution, then ½ its volume of 10 per cent caustic potash and boil. Result? (Trommer's test).

(4). Boil a little Fehling's solutions in a test tube. Result? Then add some sugar solution and boil again. Result?

3. Ferment Action: Enzymes.

a. Salivary diastase; Ptyalin. Collect a few cc. of saliva in a test tube; dilute with about five volumes of water and filter. Make the following mixtures:

1. Equal volumes dilute starch paste and saliva solution.

2. The same using saliva that has been previously boiled. Place tubes in incubator warmed to 40 C. After fifteen minutes, test each mixture for starch (iodine) and sugar (Fehling's). What effect does ptyalin have on starch? What effect does boiling have on ptyalin?

II. HYDROCARBONS (Oils, Fats).

(1) Note physical properties, differences in melting point, etc., of three fats,—olive oil, butter and tallow. Test solubilities of these fats in water, alcohol, chloroform, ether. (2) Shake a few drops of olive oil with water in a test tube. What happens? Set tube aside for a few minutes. What happens? Shake up a few drops of the oil with one per cent sodium carbonate instead of water, examine with microscope, and note difference in results. (This is an emulsion.) (3) Place a small amount of butter on a slide, and put on it a drop of 2 per cent osmic acid. Observe under microscope what occurs.

2. Chemical Tests for Fats. The reaction with ether and osmic acid are two well known tests for fats.

III. PROTEIDS (Albumens, etc.).

Use white of egg as type of proteid.

1. Carefully pour white of egg into a dish. This is approximately a 12 per cent solution of a proteid (albumen). Notice its consistency; observe that it can be drawn out into threads. Test its reaction with litmus paper; is it acid, alkaline, or neutral? Of

one half of the albumen make a 10 per cent solution. To do this, place in an evaporating dish and cut the albumen up with scissors; this frees it from membranes. Then mix with 9 times its volume of distilled water, stirring thoroughly; filter. Keep the undiluted half for further use.

2. Coagulation. (1) Coagulation by heat. Have a water bath with water at the boiling temperature. Put some of the undiluted albumen in a test tube and place in the water bath. Does it coagulate? Try a little of the 10 per cent solution in the same way. Does it coagulate? What is the effect of dilution on coagulation by heat? (2) Coagulation by chemicals. To 5 cc. of the 10 per cent albumen add a few drops of 3 per cent copper sulphate. Try also strong nitric acid and sulphuric acid, allowing a drop or two to run down the side of the test tube. Try also 95 per cent alcohol. (3) Manner in which coagulation takes place. Dip a thin thread of silk in a 3 per cent solution of copper sulphate and lay the thread on a glass slide beneath a cover glass. Allow some of the 10 per cent solution of white of egg to run under the cover while observing the operation with high power of the microscope. The albumen about the thread will be seen to form small granules appearing like a fine cloud, and these later run together and form a network.

3. Chemical Tests for Proteids. (1) *Xanthoproteic Reaction.* Dilute some of the 10 per cent albumen till it is about 2 per cent; place a small quantity in a test tube. Add a few drops of nitric acid. What occurs? Boil. What occurs as to color and other changes? Cool the solution and add ammonia until saturated. Note color produced. (This is the essential feature of this reaction.) Try in the same way a weak solution of gelatin (albuminoid); does it give the xanthoproteic reaction? Try this reaction also with water containing many infusoria; first heat the water containing the animals, then add the nitric acid and ammonia. What results?

4. Action of Enzymes on Proteids. Place thin pieces of boiled white of egg in artificial gastric juice, made by adding pepsin to a 0.2 per cent solution of hydrochloric acid; label and allow to stand till next day. Place some of this digested albumen in one dialyzer and some fresh, undiluted albumen in another; after one hour test the water below each dialyzer for albumen.

B. MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF LIVING THINGS

Living matter, or protoplasm, is not a single chemical compound, but is a combination of many albumen-like compounds. It exists only in the form of cells, individual masses of protoplasm containing a denser body, the nucleus.

I. CELL STRUCTURES.

In sections through the root-tip of an onion observe the shape and size of the cells. Having found one or more complete cells with round nucleus observe and *draw* the following parts: 1. Cell Membrane. 2. Nucleus. 3. Cytoplasm (protoplasm surrounding nucleus). In the nucleus observe: 4. Nuclear Membrane. 5. Chromatin (stained part of nucleus). 6. Achromatin (unstained part of nucleus).

II. CELL FUNCTIONS.

Each cell performs all the fundamental functions of life—it nourishes and reproduces itself, is contractile and sensitive, though some cells are devoted more exclusively to one of these functions than to the others (Specialization). In this place we study only the functions of reproduction and movement.

1. Cell Reproduction. All cells reproduce by division; the nucleus first divides, in one of two ways, after which the cell body constricts into two. Nuclear division occurs by the indirect process (Mitosis), or by the direct process (Amitosis).

(1). Indirect Nuclear Division (Mitosis). In prepared slides of the root-tip of the onion observe nuclei in mitosis, and note (a) the Spindle, (b) the Chromosomes (chromatic rods), (c) the Equatorial Plate (stage in which the chromosomes surround the equator of the spindle). The stages of division leading up to the equatorial plate are (d) the Prophase; the equatorial plate stage, during which each chromosome splits longitudinally, is (e) the Metaphase; the stage after the equatorial plate during which the halves of each chromosome separate, moving toward the poles of the spindle, is (f) the Anaphase; later stages in the division of the cell body, and the formation of daughter nuclei are known as (g) the Telophase. *Draw cells in each of these phases of division.*

(2). Direct Nuclear Division (Amitosis). In prepared slides of the follicle cells surrounding the egg of the cricket observe

various stages in the direct division of the nucleus. *Draw cells in which* (a) *the nucleolus is dividing, but the nucleus is still spherical,* (b) *the nucleus is dumb-bell shaped,* (c) *the nucleus is divided into two.*

2. Protoplasmic Movement. With a pair of fine forceps pull off some of the hairs which grow on the stamens of the flower of the spiderwort (Tradescantia) and mount them in water under a cover glass. Observe: (1) The hair is made up of a succession of cells, with corrugated walls. (2) Just within the cell wall is the granular protopasm, strands of which may be seen moving or circulating. (3) Within this protoplasm is a clear spherical or ovoid body, the nucleus. (4) Most of the center of the cell is occupied by a purple, homogeneous fluid, the cell sap.

Make a drawing showing these structures, and indicate by arrows the direction of the protoplasmic movement.

C. CLASSIFICATION OF ANIMALS AND PLANTS.

Chief Subdivisions of the Animal Kingdom.

SUBKINGDOM	DIVISION	PHYLUM (=TYPE)	SUBPHYLUM	CLASS
A. PROTOZOA Without gastric cavity, germ layers or tissues.	.....	I. Protozoa Entire body consists of a single cell which may be independent or joined with others to form a colony.	.....	1. Flagellata (Euglena) 2. Ciliata (Paramecium) 3. Sarcodina (Ameba) 4. Sporozoa (Gregarina)
B. METAZOA With gastric cavity, germ layers and tissues.	(a) *Protaxonia* (= Coelenterata) With persistent gastrular axis, and radial symmetry.	II. Spongiaria	.....	.....(Sponges)
		III. Cnidaria (Stinging Cells)		1. Hydrozoa (Hydra) 2. Scyphozoa (Jellyfish) 3. Anthozoa (Coral)
		IV. Ctenophora (Comb Bearers)	.....	.....(Jelly Spheres)
	(b) *Heteraxonia* (= Bilaterata) Chief axis not that of gastrula. Bilateral symmetry.	V. Platyhelminthes (Flat Worms)	(a) Platoda	1. Turbellaria 2. Trematoda (Flukes) 3. Cestoda (Tapeworms)
			(b) Nemertinea	
		VI. Rotifera (Wheel Animalcules)		
		VII. Nemathelminthes (Thread Worms)	(a) Nematoda (b) Gordiacea (c) Acanthocephala	(Round Worms) (Hair Worms)
		VIII. Chaetognatha (Arrow Worms)		
		IX. Annelida (Ringed Worms)		1. Chaetopoda (Bristle Worms) 2. Gephyrea 3. Hirudinea (Leeches)
		X. Arthropoda (Jointed Legs)	(a) Branchiata (With gills)	1. Crustacea (Crayfish)
			(b) Tracheata (With tracheae)	1. Onychophora (Peripatus) 2. Myriopoda (Thousand Legs) 3. Insecta (Insects) 4. Arachnida (Spiders)

SUBKINGDOM	DIVISION	PHYLUM	SUBPHYLUM	CLASS
		XI. Molluscoidea	.....	1. Phoronida 2. Brachiopoda 3. Polyzoa
		XII. Mollusca	(a) Aglossa	1. Pelecypoda (Oyster, Clam)
			(b) Glossophora	2. Amphineura (Chiton) 3. Gasteropoda (Snail) 4. Scaphopoda 5. Cephalopoda (Squid)
		XIII. Echinodermata	.....	1. Holothuroidea (Sea Cucumber) 2. Echinoidea (Sea Urchin) 3. Asteroidea (Starfish) 4. Ophiuroidea (Brittle Star) 5. Crinoidea (Stone Lilies)
		XIV. Chordata	(a) Adelochorda	(Balanaglossus)
			(b) Urochorda	(Ascidian, Sea Squirt)
			(c) Cephalochorda	(Amphioxus)
			(d) Vertebrata	1. Cyclostomata (Lamprey) 2. Pisces a. Elasmobranchii (Sharks) b. Ganoidei (Armored fish) c. Teleostei (Bony fish) d. Dipnoi (Lung fish) 3. Amphibia (Frogs, Toads) 4. Reptilia (Lizzards, Snakes) 5. Aves (Birds) 6. Mammalia (Mammals)

Chief Subdivisions of the Plant Kingdom

Subkingdom	Division	Subdivision	Class
A. Protophyta	I. Thallophyta	1. Flagellatae	Photoflagellatae (Euglena)
		2. Schizophyta	Schizomycetes (Bacteria)
		3. Myxomycetes (Slime moulds)	Cyanophyceae (Blue-green Algae)
		4. Eumycetes (Fungi, Moulds, Mushrooms, Lichens)	
B. Metaphyta		5. Euphyceae (Algae, Sea weeds, Flowerless fresh water plants)	
A'. Cryptogamia	II. Bryophyta	6. Hepaticae (Liverworts)	
		7. Musci (Mosses)	
	III. Pteridophyta	8. Filicineae (Ferns)	
		9. Equisetineae (Horsetail rushes)	
		10. Lycopodineae (Club moss)	
B'. Phanerogamia	IV. Spermaphyta	11. Gymnospermae (Cycads, Conifers)	
		12. Angiospermae (Flowering plants)	

The minor subdivisions of both animal and plant kingdoms are very numerous and are known as Families, Genera, Species and Varieties. The scientific name of any animal or plant consists merely of the name of the genus and species to which it belongs. This method of naming animals and plants is due to Linnæus (1707-1778) and is known as *binomial nomenclature*.

Inspect the specimens in the Museum, Herbarium and Vivarium, and become familiar with as many as possible of the classes named above. Enter in your laboratory notes in the following manner the scientific name (copied from the labels of specimens on exhibition) of some one member of each class and phylum of the animal kingdom, so far as represented in the exhibits:

Phylum	Class	Genus	Species
Cnidaria etc.	Hydrozoa	Gonionemus	murbachii

D. PROTOZOA AND PROTOPHYTA.

One-celled animals and plants in which the entire body consists of a single cell, which may be independent or may be joined with others to form a colony.

I. PARAMECIUM CAUDATUM.

(A Ciliate Protozoon).

A. MORPHOLOGY.

Put a small drop of water containing Paramecia on a slide; surround it with gelatin to limit the movement of the animals, cover and examine with the low power (of the microscope) and then with the high power. Note:

1. Size: measure.

2 Shape: fusiform; rounded at the anterior end, bluntly pointed at the posterior end.

3. Locomotion: due to cilia uniformly distributed over the whole surface. Note also movements of flexion.

4. Structure. The two layers: ectosarc (ectoplasm) and endosarc (endoplasm).

a. Ectosarc (Cortex): the firm elastic outer layer; its deeper part marked by oblique *myophan striations,* probably due to longitudinal wrinkling of the inner surface of the ectosarc.

(1). The cuticle, a delicate superficial layer differentiated from the underlying protoplasm.

(2). Cilia, delicate vibratile filaments arising from the ectosarc and protruding through openings in the cuticle. These openings can be seen on a specimen from which the water is allowed to evaporate.

(3). Trichocysts: minute oval sacs in deeper part of the ectosarc arranged perpendicular to the surface; when the animal is irritated, e. g. by iodine, a stiff thread can be shot out and projected beyond the cilia. They are probably defensive organs.

(4). Two contractile vacuoles in the ectosarc of the dorsal side about ⅓ of the animals' length from each end. While dilating they are nearly spherical, but at the moment of contraction separate canals can be seen radiating from them.

(5). The buccal groove begins at the anterior end of the left side and runs back to the mouth near the middle of the ventral side. The cilia of the groove drive food particles into the mouth. Run some Chinese ink in water under the cover glass and note that some of it is carried into the groove.

(6). The mouth is an aperture in the ectosarc at the posterior

end of the groove through which food passes into the endosarc. Watch the ink collect at the inner end of the gullet into a ball which is suddenly passed into the endosarc. Watch the course of the food ball around the body until it is finally ejected.

(7). The anus is a temporary aperture between the mouth and the hinder end of the body—visible only at the moment of ejection of fecal matter.

b. The Endosarc (Medulla) is the more fluid protoplasm filling the central portion of the body.

(1). The food vacuoles are spherical spaces in the endosarc filled with water containing food particles.

(2). The circulation of the endosarc is rendered obvious by the food vacuoles and the granules, which are carried round in a definite direction.

(3). The nucleus is an elongated oval body near the center of the body of the animal. It is best seen with light reflected from the slide, not from the mirror, or in stained specimens.

(4). The micronucleus is a much smaller body applied to one side of the nucleus and resembling it in appearance.

Make one or two drawings of an animal to show the above mentioned structures.

B. PHYSIOLOGY.

I. Metabolism.

1. Ingestion of food. Place some Paramecia on a slide with powdered Chinese ink and watch the formation of food balls in the gullet and their ingestion. Study the formation of a food vacuole.

2. Circulation of Endosarc. Observe and sketch the changes of position of the food vacuoles in the body and show by arrows the course of circulation. Time the circulation by noting the time at which the ink is added and that at which the first ink ball completes the circuit.

3. Nature of food. Study the nature of the contents of the food vacuoles of normal Paramecia and find, if possible, what they feed upon. Is it animal or vegetable matter? Does Paramecium choose its food? Stain with iodine and see if any starch is used as food.

4. Digestion. Observe the changes in color, etc., of food as it passes through the body, also the changes in the vacuoles and their fluid contents. What do these changes indicate?

5. Egestion. Observe, if possible, the egestion of ink from a

food vacuole, and from the body. *Show by a drawing where and how this takes place.*

6. Excretion. The contractile vacuoles are excretory organs for getting rid of water and nitrogenous waste (urea). Study and *sketch* a vacuole in various stages of contraction and expansion. Time the contractions and expansions and record the results in your notes. Place Paramecia in a thick solution of Chinese ink and observe the extrusion of a clear drop of fluid at the moment of contraction of the contractile vacuole.

7. Respiration. Place a number of the animals in a drop of phenolpthalein (which loses color in the presence of carbon dioxide); also note the manner in which the animals collect at the surface of a dish in which the water is very foul. What do these observations teach? Are there any organs of respiration?

II. Reproduction.

1. Fission. Observe and *draw* a Paramecium in the process of division. In a stained preparation note what changes take place in the nucleus and micronucleus during this process. Can you detect any difference between the two daughter individuals? How do the contractile vacuoles, the buccal grooves and the gullets arise in the two?

2. Conjugation. Study and *draw* living individuals in the act of conjugation. What portions of the body are in contact? Is there any distinction of size or sex in the two individuals? In stained preparations study the nuclear changes which take place during conjugation. *Draw.* Isolate an individual in a watch glass full of water and note how many individuals are present on the next day.

III. Irritability.

1. Automaticity. Does the animal appear to act of its own accord or only through the influence of external stimuli?

2. Movement. How many kinds of movement does the animal exhibit? What are the organs of locomotion? With a dissecting microscope observe the movements of an animal in a drop of water. Does it move in straight lines? Does it keep one side uppermost? How does it alter its course? Can it move backwards? By means of lines and arrows plot the movements of an animal during one minute. By means of powdered ink observe the direction of currents over the body. What is the direction of the currents in the buccal groove?

3. Sensitivity. Is the animal sensitive to touch or pressure? How

does it behave when in contact with a solid body? Place a small drop of salt solution colored with Chinese ink on a slide and note whether the animals are sensitive to this substance. In similar manner test them with 1-10 per cent and 1-2 per cent acetic acid, and also with a bubble of carbon dioxide. Place animals on a slide heated at one end and cooled at the other. What results? In a similar manner test them with the electric current and record your results. Also test their sensitivity to light and gravity.

II. VORTICELLA SP.

(A Ciliate Protozoon).

A. MORPHOLOGY.

Examine filaments of algae, etc., for bell-shaped organisms borne on a stalk. If a single individual is borne on a contractile stalk it is some species of Vorticella; if many individuals form a colony on a common branched stalk it is some species of Carchesium or Zoothamnium (in Zoothamnium the stalk contains a common muscle fibre which branches with the stalk); if the stalk is branched but not contractile it is some form of Epistylis. Which genus are you examining?

Study under high power and note:

I. The Stalk. 1. Shape; length compared with width. Shape, when contracted and expanded. If branched, is there any regular arrangement of the branches?

2. Structure. Is there any distinction in the stalk between ectoplasm and endoplasm? The central axial filament or contractile fibre is easily seen. Does it run in the exact center of the stalk? Does it join the ectoplasm or the endoplasm at its upper end? With your highest power study the structure of the axial filament.

II. The Body. 1. Shape, when seen from above; from the side. What is its shape when contracted? When expanded? In a fully expanded individual observe:—(a) The peristome or rounded edge of the bell. Does it extend completely around the disk? (b) The disk, a lid-like structure within the peristome raised on one side. (c) Between the peristome and the disk a ciliated depression leading down under the raised portion of the disk into a deep pit, the vestibule. Is there anything corresponding to this in Paramecium?

2. Structure. Observe the following: (a) A transparent layer covering the body,—the cuticle. (b) Within the cuticle a clear cortical layer, the ectoplasm. Where is it thickest? (c) A central

granular mass, the endoplasm, containing food vacuoles, etc. (d) A contractile vacuole is present just below the disk. Note its shape. (e) The nucleus, an elongated cylindrical body. Is it in the ectoplasm or endoplasm? (f) The oesophagus or gullet, a ciliated tube leading down from the vestibule. (g) Anus, an opening into the side of the vestibule, to be seen only at the moment of egestion of food remnants.

B. PHYSIOLOGY.

I. Movement.

a. General movements. Study the manner in which the stalk contracts? What part of the stalk causes the contraction? What change takes place in the body during contraction? Note the rapidity of the movements. How does the animal assume the expanded condition? What causes it? What part is the first to assume the expanded state? Compare the rate of contraction with that of expansion. Note that the body sometimes separates from the stalk. How does the body move after separation?

b. Ciliary movements. Add powdered Chinese ink to the water and observe the direction of the currents produced and the method of feeding. What is the significance of the animal's scientific name? How many kinds of cilia does Vorticella have? To how many uses are they put? How does it differ from Paramecium in this respect?

II. Reproduction.

1. Fission. Look for animals undergoing fission. Where does the division begin? By the aid of stains study the nuclear phenomena of division. 2. Conjugation. Individuals may sometimes be seen undergoing conjugation. Study the process and compare with the conjugation of Paramecium.

Make drawings of all you have observed.

III. STYLONYCHIA MYTILUS.

(A Ciliate Protozoon).

In the same sort of material in which Paramecium is found look for a broad, flattened infusorian, which moves swiftly about. This is Stylonychia. Compare its size with that of Paramecium.

Notice two sorts of motion: a rapid swimming as in Paramecium, and a crawling by means of the large ventral cilia.

Structure. Note the ectosarc and endosarc, the former contain-

ing no trichocysts, and less distinct than in Paramecium: two nuclei about one third of the animal's length from either end: one contractile vacuole: a buccal groove, much like that of Paramecium; along the left side of the groove a row of long powerful cilia. Three groups of gigantic cilia on the ventral surface. The cilia of the anterior and middle groups are hook-like, those of the posterior group paddle-shaped. Along the margin of the ventral side is a row of large cilia, three of which project posteriorly as long bristle-like processes. Observe the method in which all three sorts of cilia are used.

Make a drawing to show all the structural features of Stylonychia.

IV. AMOEBA PROTEUS.

(A Rhizopod Protozoon)

Place a small drop of sediment from a vessel containing Amoeba on a slide with a drop of water; cover with cover glass and search for Amoeba with low power. If not easily found, prepare several such slides and examine them after they have been standing for some minutes, so that the Amoebae may crawl out of the sediment. When an Amoeba is found examine with a high power and note:

A. MORPHOLOGY.

1. Size; is it visible to the naked eye?

2. Shape; is it regular? Constant? Are the pseudopodia of the same size and shape? Do they ever branch? How many do you find? Sketch at intervals of one minute for five minutes.

3. Structure: An outer clear layer, the ectosarc, and an inner granular more opaque substance, the endosarc (endoplasm). Is the boundary between the two layers a sharp one? Where is the ectosarc thinnest? Is there a membrane outside this layer? Are all the granules of the endosarc of the same size? Which layer is the more fluid? In the ectosarc a clear vesicle may be found which appears and disappears; this is the contractile vacuole. How long does it take it to contract, how long to expand? Are there any visible contents? Is there more than one contractile vacuole?

In the endosarc a round, clear body may be found, which does not change shape; this is the nucleus. Is it more solid than the surrounding protoplasm? What is its shape? Size? Is it always in the same place? There are often also in the endosarc various foreign bodies which serve as food, such as diatoms, desmids, green cells, etc. *Draw to show structure.*

4. Life Cycle: Look for various stages in the life history; encysted specimens, small individuals, large ones, etc. Also note various kinds of Amoebae, some with short lobe-like pseudopodia, others with longer slender ones; some large and coarsely granular, others small and finely granular.

5. Stained Specimens: In a prepared specimen, stained and mounted, observe nucleus, ectosarc, endosarc, etc. *Draw.*

B. PHYSIOLOGY.

1. Movements: Is motion continuous? Regular? How is it produced? Watch process of formation of a pseudopodium. What part does the ectosarc play in the process? The endosarc? Watch an active Amoeba and trace on paper its path of motion. Are there permanent anterior and posterior ends? Does there seem to be any difference in surface tension between the anterior and posterior ends? Are the currents in the endosarc constant? Indicate in a drawing the course of the currents by arrows. Where are the currents swiftest? Where slowest? Are cilia present on any portion of the body?

2. Nutrition: If possible watch the process of taking in food and of its egestion. What does the animal eat? How and where does it take in food? Are food vacuoles formed? Is there a definite course of circulation of food within the body? Where is the food digested? How distributed? How are gaseous, liquid and nitrogenous waste substances expelled from the body?

3. Reproduction is difficult to observe and may be omitted from notes.

4. Irritability: Are there any indications that Amoeba is sensitive to mechanical stimuli? Is it sensitive to chemicals? To changes in temperature? To electricity? To light?

Does Amoeba show any reflex movement? Is it behavior more or less varied than that of Paramecium.

Make careful drawings and notes of all that you have observed.

V. EUGLENA VIRIDIS,

(A Flagellate).

Place a drop of water containing Euglena on a slide and after covering look with the low power for green spindle-shaped organisms which swim swiftly. Having found them study with the high power and note: (1) Size. (2) Color, due to chlorophyll. The anterior end is colorless. Near the anterior end is a red pig-

ment spot, the stigma. (3) Shape, fusiform; the anterior end is blunter than the posterior and bears a long flagellum which may be lost in some specimens. The flagellum arises from the bottom of the gullet, or mouth opening. Observe the contractile vacuole, nucleus and the paramylum bodies near the center of the body. What color do the paramylum bodies take when stained with iodine? What does this indicate? Is there any cell sac?

Look for animals in the encysted condition, showing stages in division. Determine by the use of Schultze's solution whether or not there is cellulose in the cyst.

Movements are of two kinds: (a) Rapid swimming movements in which the flagellum is carried forwards. (b) Worm-like movements, contractions and expansions by which the animal crawls about. The latter movements are characteristic of Euglena and are called "euglenoid" movements. Draw at intervals to show changes in shape.

Make drawing to show all that you have observed.

VI. SPHAERELLA (HAEMATOCOCCUS)

(A Flagellate Protophyte).

A. RESTING STAGE.

(1). Spread out in water some sediment containing Sphaerella, put on a cover glass and look with low power for red or green spheres. Having found one examine with high power and note:

(a). Size, variable; draw several to scale.

(b). Form; spheroidal.

(c). Structure; a sac surrounding the contents which consist of protoplasm, chromatophores, a nucleus and sometimes a vacuole.

(d). Color; red, green or partly one and partly the other. Where is the coloring matter always situated?

(2). Place a drop of iodine solution on the slide at the edge of the cover glass, apply a bit of blotting paper at the other side, thus drawing the iodine solution under the cover. What parts stain? How does it affect the nucleus and the chromatophores?

(3). Look for individuals in the process of division, some elongated with transverse lines of division, others divided into two or more smaller portions often lying within the sac of the parent Haematococcus. *Draw various stages in this division by fission.*

B. MOTILE STAGE.

(1). After dried, resting forms have been in water for twelve hours examine for motile forms and note their movements.

(a). An active transition from place to place.

(b). A rotary motion around the long axis.

(2). Note the following kinds of motile forms:

(a). Large individuals, the *macro-zoospores,* of the same size as the resting forms, each surrounded by a thin colorless cell wall, separated from the contents by a clear space.

(b). Much smaller motile forms, each surrounded by no separate cell wall, but with two cilia or flagella at the pointed end or "beak". These are the *micro-zoospores.*

(3). In the larger forms note: Color, structure, contents, sac (cell wall), cilia, protoplasmic bridles. Which end goes ahead in swimming? How are the contents held in place within the sac? In an individual which has nearly ceased movements study the successive positions assumed by the flagella, and their mode of bending to and fro. Treat with iodine: the protoplasm is killed and the cilia are rendered conspicuous.

Draw individuals in motile stages to show all of the above mentioned points.

VII. VOLVOX GLOBATOR.

(A Flagellate Protophyte).

Place a drop of alcohol containing Volvox on a hollow-ground slide cover and examine with low power. Having found a colony as perfect as possible, observe with high power and *draw* the following:

1. Form; spherical when not disturbed.

2. Size; variable depending upon age of colony.

3. Structure; a hollow sphere surrounded by a single layer of cells (zoöids) each of which in life bears two flagella and contains chlorophyll, a nucleus and a stigma or eye spot. The zoöids are connected together by a gelatinous substance, and each is connected by strands of protoplasm to the zoöids immediately surrounding it.

4. Life Cycle; observe the cells which migrate into the cavity of the sphere from the outer wall. These are the sex cells, which are of two kinds: a. Parthenogonidia, which without fertilization give

rise to small colonies by repeated divisions; b. Ovaries, each of which contains a single ovum surrounded by a spinous membrane; c. Spermaries, each of which contains many spermatozoids. After a spermatozoid has entered an ovum the latter undergoes repeated divisions and gives rise to a daughter colony.

Draw various stages in the formation of the daughter colonies from parthenogonidia and from fertilized ova.

VIII. SACCHAROMYCES CEREVISIAE

(A Fungus).

A. MORPHOLOGY.

Place some growing yeast in a drop of water on a slide and examine under a low power, then under the high one. Observe the small oval bodies or yeast cells. Note: Size; is it constant? Measure several. Shape; does it change? Nature of surface. Mode of union. Is there any regular number or arrangement of cells in the various groups? How many cells in a complete yeast plant? Structure: Observe the cell wall; contents. Is a vacuole present? Where is it found? Do you ever find more than one? Is it contractile? A nucleus is present but it can be demonstrated only by the most careful staining. Place a piece of blotting paper over the cover glass and press firmly upon it; in this way some of the cells will be bursted and the sac and contents can be studied separately. (a). What is the nature of the sac? Its color? Is the color of the cell due to the cell wall or contents? Is there any opening in the sac through which food can be ingested? Are there any organs of locomotion? Is the wall elastic? (b). What is the physical nature of the contents? Its color?

Draw several cells to show size, mode of union and structure.

B. CHEMICAL REACTION.

1. Run a drop of aceto-carmine under the cover glass and observe which individuals stain soonest and most deeply. Do the crushed cells stain as readily as the entire ones? Does the sac stain?

2. Treat another drop of yeast with dilute caustic potash. What happens to the cells?

3. Kill some yeast cells by boiling them with water in a test tube. Mount some of this dead yeast and stain with aceto-carmine. Does it stain differently from the living yeast? What inferences may be drawn?

4. Mount a fresh drop of yeast on a slide and treat with a drop of iodine. What is the effect on the cells? Is starch present in the fluid? Is there any starch in the cells themselves?

Make drawings of the yeast cells showing the effect of the reagents.

C. PHYSIOLOGY.

In the following experiments the amount of growth which has taken place may be roughly measured by the increase of the turbidity in the liquid. It may be tested microscopically by the number of buds to which the cell has given rise.

1. Effect of food supply upon growth. Take five test tubes each one-third full of the solution named: (a) distilled water; (b) 10 per cent solution of sugar in water; (c) Pasteur's solution without suger; (d) Pasteur's solution with sugar; (e) Mayer's pepsin solution.

Carefully label each tube and put a drop of yeast into each; shake the tubes thoroughly and tightly plug the mouth of each with a wad of clean absorbent cotton and set them in the incubator, heated to 35 degrees C, for two or three days. Examine the tubes from day to day and judge, from microscopic examination and the turbidity, in which fluid the yeast grows best. In which are the most bubbles of gas formed? Does the formation of gas bear any relation to the growth?

2. Reproduction. (a) Budding. With the microscope examine cells from each of the test tubes. In which have the cells the largest number of buds? In which the smallest? How many buds may a cell have? Show by drawings the steps in the formation of a mature bud. What is the difference between budding and fission? (b) Endogenous Spore Formation may sometimes be observed in yeast which has been kept for sometime under unfavorable conditions, e. g. lack of food, moisture, etc.

3. The effect of growth of yeast upon food supply. (a) Taste of the 10 per cent sugar solution after yeast has been growing in it for a day or two. Compare with a solution in which there is no yeast. How do you explain the difference? (b) Examine the distillate of a solution of sugar in which yeast has been growing for a day or two. Notice that it has the taste and odor, and burns with a pale blue flame, characteristic of alcohol. (c) Nature of the gas given off. Take two test tubes, fill the first ⅓ full of clear baryta water, fill the second about ½ full of yeast which is actively giving off bubbles of gas. Insert a cork in this second tube and

connect the two by a bent glass tube one end of which passes through the cork into the air space above the yeast, the other end of which dips below the surface of the baryta water. What changes take place in the baryta water? This is a test for carbon dioxide. (d) Chemical reaction of fluid yeast. Determine by the use of litmus paper whether fluid yeast is acid or akaline in its nature. What do you suppose the cause of this to be?

Prepare a written statement giving as far as possible an explanation of all the facts you have observed in these experiments.

IX. BACTERIA SP.

(Schizophytes).

A. BACTERIA OF HAY INFUSION.

Fill three test-tubes ½ full of a fresh infusion of hay: (1) close one tube with cotton wool carefully and boil a few minutes; (2) do the same with a second tube and then boil it again after 24 hours and repeat the boiling for several days if convenient; (3) leave a third tube open and do not boil it; set all three in a warm place where they can be observed from time to time. Note the changes which take place in each of the tubes. Does the infusion in any of the tubes become turbid and in which one is this most marked? Determine by microscopic examination what the cause of the turbidity is. How do you account for the differences between the tubes? Keep the tubes under examination for several days or weeks and observe in what tubes a scum forms on the top of the liquid. Does the formation of this scum have any influence on the turbidity of the fluid? Study the scum under the microscope and determine what it consists of and whether it differs in the different tubes. What ultimately becomes of the scum? What changes if any are there in the odor of the fluid during the period of observation and how do you account for them? Are different kinds of bacteria found in the tubes? If so make sketches to show them. Do the bacteria in the same tube differ in form from day to day? If so sketch them in the order in which they appear. After one week what is the condition of the fluid and the bacteria found in each of the tubes? *Write up an account of the phenomena you have observed and give your explanation of them.*

B. BACTERIA OF THE AIR.

Clean a smooth potato with a stiff brush and water, removing with a knife all injured portions as well as the "eyes". Sterilize the

potato in boiling water for thirty minutes, then cut it in slices by means of a knife sterilized in a Bunsen flame. Place the slices of the potato on a sterilized glass plate and leave the clean cut surface exposed to the air in the room for one hour. Cover with a sterilized bell jar under which some distilled water is placed to maintain a moist atmosphere and set aside for several days. If any organisms develop on the potato they must have come from the air of the room. Observe on the potato variously colored spots or "colonies". Are all of these colonies bacteria? Are all the organisms in a colony alike? What is the significance of this fact?

C. BACTERIA OF HYDRANT WATER.

Take a sterilized gelatin culture plate in a Petrie dish (each locker is supplied with one) open the dish and quickly allow a few drops of hydrant water to run across the gelatin. Close the dish at once and set it aside for several days. If bacteria were present in the water one or more colonies of them will be found along the path of the drop. Study these colonies as in the preceding experiments.

D. BACTERIA OF MILK.

Dilute 1 cc. of milk with 100 cc. of sterilized water. Add 1 cc. of this dilution to a Petrie dish of "litmus agar", cover and place in the incubator for 36 hours. Then by means of a "counting plate" determine the approximate number of colonies present in the agar, and calculate the number of bacteria present in 1 cc. of undiluted milk. Colonies which have the form of minute footballs belong to the group of "colon bacilli" and come from the intestinal tract of some mammal. What does the changed color of the litmus agar indicate?

E. BACTERIA FROM THE MOUTH.

Take some scrapings from the teeth, dilute with water, mount and study the various forms under a high power. How many kinds of bacteria can you find?

F. MOTILE STAGES.

Study bacteria from various media, viz.: Pasteur's Solution, Beef Tea, Infusions of Hay and Peas, Potato and Gelatin Cultures, Sewage, etc., and observe and *draw* the following forms:

1. *Micrococcus;* rounded forms occurring singly or in bead-like rows; without flagella.
2. *Bacterium;* short and thick rod-like forms.
3. *Bacillus;* long thread-like forms.

4. *Vibrio;* like Bacillus but with bent joints.

5. *Spirillum;* elongated threads rolled up into a more or less perfect spiral.

6. *Spirochacta;* like Spirillum but longer and more closely rolled, and without flagella. In these various forms observe the following points:

First, size, measure.

Second, structure. Can you notice any change of form in an individual? Any difference between the external and internal portions? Any peculiarity of the ends in the longer forms?

Third, movements. Some vital, others purely physical (*Brownian movements*). The former progressive, the latter vibratory around a stationary center. Study the Brownian movements in particles of Chinese ink in water. Put a few drops of fluid containing bacteria on a slide, hold the slide over a Bunsen flame and kill the bacteria by boiling, cover and examine with high power. Can you notice any movement of the dead bacteria? Compare with movements of living ones.

G. RESTING STAGES.

Examine the scum ("*Zoogloca*") from the surface of various liquids, especially the hay infusion; it consists of myriads of bacteria in a resting condition imbedded in a gelatinous substance.

H. CHEMICAL TESTS.

Spread a small drop of liquid containing motile bacteria on a clean cover glass and let it dry slowly; then pass the glass through a Bunsen flame two or three times to coagulate and fix the bacteria upon the glass. Put a drop of Methylen Blue or Gentian Violet upon the glass. After five minutes rinse with distilled water and mount in a drop of water upon a slide. If a permanent mount is desired thoroughly dry the glass after rinsing and mount in Canada Balsam.

Treat some of the Zoogloea in the same way and observe that the bacteria stain more deeply than the substance in which they are imbedded.

I. STUDY PREPARED SLIDES OF PATHOGENIC BACTERIA.

J. DEMONSTRATION OF BACTERIA SEEN WITH "DARK FIELD ILLUMINATION".

E. METAZOA.

Metazoa are many-celled animals in which there is a differentiation into at least two body layers, the *Ectoderm* and the *Endoderm;* the former is the organ of relation, the latter the organ of nutrition; in addition all have heteromorphic sex cells, ova and spermatozoa. In all metazoa the fertilized ovum undergoes repeated divisions (Cleavage) which lead up to the formation of a hollow sphere of cells (Blastula) and from the latter arises a two layered condition (Gastrula) the outer layer being the *Ectoderm*, the inner the *Endoderm;* between these two a third layer, the *Mesoderm*, is usually formed.

The cells of the different germinal layers differ from one another, and in the course of further development differentiations appear among the cells of the same layer. In this way arise Tissues, differentiated groups of like cells and their products. From the two primitive tissues, epithelium and mesenchyme, present in the blastula and gastrula all other tissues are derived, as shown herewith:

1. Epithelium	2. Mesenchyme
a. Epithelial tissue	a. Connective tissue
b. Muscular "	b. Skeletal "
c. Nervous "	c. Vascular "
d. Germinal "	d. Storage (reserve) "

These different tissues will be studied in the laboratory in connection with each animal considered.

The various functions of animal life, which in the Protozoa are all performed by a single cell, are performed in the Metazoa not only by many cells and tissues but also by groups of different tissues united to form Organs, each with a specific function, and by groups of organs united to form Systems, each having some one general function, as shown in the following table:

Functions		Organs	Systems
I. Metabolism	Ingestion, Digestion, Egestion	Mouth, Teeth; Stomach, Intestine; Anus	Alimentary
	Respiration	Trachea, Lungs, etc.	Respiratory
	Excretion	Kidneys, Bladder, etc.	Excretory
	Circulation	Heart, Arteries, Veins, etc.	Circulatory
	Assimilation, Growth, Dissimilation	Have no special organs or systems	
II. Reproduction	Asexual	No special organs or systems	
	Sexual	Ovaries, Testes, Uterus	Genital
III. Irritability	Reception of Stimuli	Sense Organs	
	Transmission of Stimuli	Nerves	Nervous
	Coördination	Gangalia, Brain	
	Movement	Muscles	Muscular

These various organs and systems will be considered in detail in connection with each of the animals studied.

I. DEVELOPMENT OF OVUM.

In prepared slides of Echinoderm eggs observe the following stages:—1. *Cleavage;* 1-cell, 2-cells, 4-cells, 8-cells, 16-cells, 32- or 64-cells. Observe the appearance of a cleavage cavity after the 8-cell stage.

2. *Blastula*:—Observe the hollow sphere composed of a single layer of cells (Epithelium). Are there any indications that scattered cells (Mesenchyme) migrate into the cavity of the blastula (Blastocoel) as in Volvox?

3. *Gastrula*:—Note the flattening and ultimate infolding of the blastula at one pole. Do the cells at this pole differ in appearance from the others? The infolded cells constitute the endoderm, the outer layer the ectoderm. The infolded cavity is the *Gastrocoel,* or digestive cavity; the opening to the exterior is the *Blastopore.*

Draw and label the stages and structures named above.

II. HYDRA VIRIDIS OR FUSCA.

(Freshwater Hydrozoa).

A Metazoan which throughout life remains in a two layered condition, like a gastrula. Observe with naked eye, or with pocket lens, the hydras in a jar of water where they have been undisturbed for some time. Notice the general habitus of body, method of obtaining food, etc. Transfer a hydra to a slide with plenty of water, and observe with the dissecting microscope; afterward cover, supporting the cover glass so as not to crush the animal, and examine with the low power of the compound microscope.

I. Form.

The body: What is the general shape? Do its length and breadth vary? It is usually attached at one end, the foot, by a kind of sucking disk and terminates at the other in a conical projection, the hypostome, with the mouth at its summit. The mouth is a small aperture but it can be greatly dilated to take in food. It opens into a central cavity, the enteron (digestive). The tentacles are hollow processes of the body wall. How many are there? Compare the number of tentacles in brown and in green hydras. Is there more than one circle of tentacles? Observe the knob-like swellings on the tentacles. Measure the length of the tentacles when expanded; when fully contracted. For what purpose are the tentacles used? Buds:—Young hydras of various sizes and stages of development may be attached to the sides of the parent. Are colonies formed by budding? Why?

Draw an entire animal, with all the parts named above.

II. Structure.

1. The body wall of the animal is composed of two layers of cells, one within the other. (a) The *Ectoderm* is the outer layer. What is its color? How much of the thickness of the body wall does this layer form? (b) The *Endoderm* is the inner lining of the body cavity (digestive cavity). In the green species (Hydra viridis) it contains chlorophyll bodies; in the brown species, H. fusca, it contains "sooty corpuscles". Which layer is the thicker? (c) The supporting layer or *Mesoglea* is a thin gelatinous layer between the ectoderm and the endoderm.

2. The tentacles. Examine a tentacle with the high power. Of how many layers is it composed? Focus up and down so as to obtain views (optical sections) at various levels. Is the tentacle

hollow or solid? The elements of the two layers can be most easily seen in the tentacles. Observe the following:

(a) The ectoderm cells are large and conical with their apices directed inward. The boundaries of the outer ends form a mosaic, their inner ends rest directly on the supporting lamella. Do these cells vary in shape when the tentacle is extended or contracted?

(b) The interstitial cells are small rounded cells placed between the inner ends of the large ectodermal cells.

(c) The cnidoblasts or "thread cells" are modified interstitial cells prolonged at the outer end into a cnidocil or "trigger" and containing an oval, highly refractive capsule, the nematocyst. The capsule is filled with fluid and contains a spirally wound filament formed by the doubling in of the wall of the capsule at one pole. The nematocysts form knob-like swellings on the tentacles. They are of two kinds: (1) smaller, more numerous ones situated at the bases of the longer cnidocils and containing short stout threads; (2) larger ones lying near the middle of each knob-like swelling, globular in shape when seen from the face, flask-shaped when seen from the side; they contain long slender filaments armed with barbs at the basal end. Run in a little iodine and observe the ejection of the threads of the nematocysts. Note that the threads are turned inside out in the process of discharge, the basal portion being discharged first. What is the use of the barbs? The hollow thread? The fluid in the cysts? Do nematocysts occur anywhere else than on the tentacles?

(d) The endoderm cells line the cavity of the tentacles. They are large and some of them bear flagella by which currents are caused. Focus on the middle of the thickness of a tentacle and observe the flagella on the endoderm cells and the nutrient particles streaming up and down the cavity of the tentacle. What difference can you detect in the relative numbers of these elements (cells) in the various parts of the body?

Make drawings to show the characteristic layers, and cellular elements.

III. Reproduction.

1. Asexual reproduction occurs by the formation of hollow outgrowths from the sides of the body wall. Each of these acquires a mouth and tentacles at the distal end of its body and finally, constricting at the base, separates from the parent animal. Look for such buds in various stages of development.

2. Sexual reproduction: hydra is monoecious (hermaphroditic), the same animal producing eggs and spermatozoa.

(a) The spermaries are swellings of the body wall produced by the local multiplication of interstitial cells, and covered on the outside by a cap formed of large ectodermal cells. The spermaries are situated just below the tentacles. How many do you find? Is the number constant? Find a ripe spermary and observe the movement of the spermatozoa within the capsule. By gentle pressure upon the cover glass break open the capsule and observe the swimming of the spermatozoa and their size and shape.

(b). The ovaries usually develop later than the spermaries and are formed near to the base of the animal. How many do you find? Is the number the same in the brown and green species? Single cells of each ovary enlarge to form the ovum, while the other cells nourish it and form a capsule about it.

Make drawings of buds and of the sexual organs.

IV. Study of Prepared Sections.

Examine series of transverse and longitudinal sections of hydra prepared by the paraffin method, and note the large central enteron surrounded by a body wall of two layers of cells.

1. The ectoderm. Is it of uniform thickness? In it observe: (a) Large squarish or conical cells. Do they contain nuclei and vacuoles? Their basal ends are continued into muscle fibres (Klinenberg's Fibres) which are mainly longitudinal in direction, and in cross-section appear as a row of refractive dots on the surface of the supporting lamella. Over the outer surface of these cells is a thin cuticle. At the foot the ectoderm cells are more columnar and contain granules. (b) Interstitial cells are present over the body and tentacles but absent in the foot; they stain deeply. (c) Nematocysts, abundant in the tentacles, less numerous on the body and absent on the foot. Are they found in the endoderm?

2. The supporting lamella. A thin, deeply staining layer between the ectoderm and the endoderm. Is it composed of cells?

3. The endoderm cells; variable in shape and size. They are of two kinds: (a) Larger cells, irregular in shape and size, containing vacuoles, and with the nucleus flattened and near the basal end. In H. viridis the basal part of each cell contains rounded bodies, chloroplastids, coated with chlorophyll. In H. fusca similar bodies are present, "sooty corpuscles", devoid of chlorophyll. The basal ends of these cells are often prolonged into muscular processes like those of the ectoderm cells, but transverse in direction. (b) The

smaller secretory cells, pear-shaped and lying between the bases of the larger ones. These last mentioned cells are numerous in the walls of the hypostome but fewer elsewhere. Their protoplasm is granular and they stain deeper than the larger cells.

Make a drawing of each section.

V. Study of Isolated Cells.

Place living hydra on a slide, draw off the water and cover for a few minutes with a drop of Haller's Fluid. Cover and tap gently upon the cover glass to separate the cells.

Select and draw good examples of the varieties of cells mentioned above.

III. HYDRACTINIA OR PODOCORYNE SP.

(Marine Hydrozoa).

Examine with dissecting microscope a colony of Hydractinia on a shell and note the way in which the shell is incrusted by the roots or base (hydrorhiza) of the colony, while the free portions, or stems (hydrocaulis) arise from this base.

With the compound microscope examine mounted preparations of Hydractinia, and observe the following: isolated polyps or zoöids which have been torn free from the base. Among these are (1) Hydranths or nutritive zoöids with mouth opening surrounded by circle of tentacles. (2) Blastosyles or reproductive zoöids, without mouth opening, with rudimentary tentacles, and with sex buds (gonophores) in which ova and spermatozoa are found. (3) Dactylozoöids, or fighting polyps, without mouth or tentacles, but with long slender body beset with nettle cells.

Observe the cell layers characteristic of Coelenterates.

Make drawings of all that you have observed.

IV. PENNARIA TIARELLA. (Marine Hydrozoa).

Observe the form and manner of branching of the colony. Which is the oldest polyp in the colony? Which branch is the oldest? How many kinds of zooids do you find?

In a stained and mounted specimen, observe the following:

(1) The stem (hydrocaulis) containing a central gastro-vascular cavity; surrounded by two layers of cells characteristic of coelenterates; the perisarc or cuticular covering, with rigid construction.

(2) The hydranth (nutritive zoöid). How many circles of

tentacles? How many tentacles in each circle? Which are the longest? Are the tentacles hollow as in Hydra?

(3) Medusoid buds, and medusae. Observe on sides of hydranths various stages in formation of medusa buds. Where are they attached? Observe the structure of a fully formed bud, and of a free medusa.

Record your observations in labelled drawings.

V. GONIONEMUS MURBACHII. (Hydro-Medusa).

The three typical layers of the Coelenterata (ectoderm, endoderm and mesoglea) are represented. The mesoglea, however, has become very thick. The bell-shaped medusa bears a large number of tentacles from its margin.

In the center of the bell is the manubrium, which bears on its free end the mouth, the latter having tentacular folds. The opening of the bell is partly closed by the velum.

The digestive system consists of a short oesophagus in the manubrium leading into the stomach; from the latter extend four radial canals to the margin of the bell. At the margin, these radial canals open into a marginal or ring canal.

The sexes are separate and the reproductive organs, both male and female, are suspended from the radial canals. Fertilization takes place in the water.

VI. PLANARIA SP. (Turbellarian Worm).

I. External Anatomy.

In the living worm distinguish between the anterior and posterior ends, and the dorsal and ventral sides. Place an animal on a slide with a drop of blood and observe the manner in which the blood is sucked up and fills the digestive tract. Then place a fresh drop of water on the animal and cover with a heavy cover glass. Observe that the animal is covered with cilia. In the following description make out as much as possible in the living animal, and supplement this with a study of stained and mounted specimens, both entire mounts and sections.

II. The Digestive System.

The mouth will be found at the extremity of a movable proboscis which hangs from the mid-ventral line. The cavity of the pharynx leads into that of the red-colored, branched intestine. The intestine is seen to be made up of three divisions (Triclad type). One division extends anteriorly and nearly reaches the dark colored eye

spots, while the other two extend laterally and posteriorly. There is no anus.

III. The Reproductive System.

The two sexes are united in the same individual, the common sexual opening being seen in the mid-ventral line a little posterior to the proboscis. The ovaries lie in the anterior portion of the body, one on either side. The ducts may be seen leading from them toward the external opening. Yolk glands appear as lobulated structures lying between the ramifications of the intestine. The testes are rounded glands scattered along the sides of the body from the anterior to the posterior ends. Do you find ducts leading from them? Along each side of the proboscis sheath is a tubule, the *vas deferens* which runs into the common sexual opening.

IV. Nervous System.

In favorable specimens the nervous system can be easily followed as a series of whitish lines and areas. The two eye spots mark the situation of the cerebral ganglia, each ganglion appearing as an irregular mass which sends a nerve posteriorly, and several smaller ones anteriorly, and laterally. The right ganglion is connected with the left by a commissure. Follow the lateral nerves posteriorly; they will be found to unite at the hinder end of the body. Are the lateral nerves above or below the branches of the alimentary canal? Study sections through the eye spots.

V. The Muscular System.

The muscular system is especially developed in the region of the pharynx. Muscle fibres also occur under the integument, the outermost layer consisting of circular fibres while there are two inner layers of longitudinal fibres separated by a layer of parenchyma. Many diagonal fibres are also found running from the dorsal to the ventral side.

VI. Excretory System.

Along the sides of the body parallel to the longitudinal nerves is a branched system of very small and clear tubules, the water vascular or excretory system. These tubules open to the exterior at many points along the dorsal surface and are difficult to trace.

Study prepared sections and *make drawings of all you have observed.*

VII. DIPLODISCUS SP. (A Trematode Worm).

1. Crush and remove the shell from an infected snail (Goniobasis) and observe the multitudes of parasites in the liver. Place the snail in a watch glass of water, and after puncturing the liver with a needle observe the escape of the parasites into the water. Among these the following stages may be observed:—(a) Sporocysts; irregular hollow sac-like structures containing germ cells (eggs), germ balls (many celled embryos), or rediae. (b) Rediae, elongated sacks with mouth and rod-shaped intestine, beginning at a pharynx in front and ending blindly behind. Near the posterior end of the body there are two processes extending out from the sides of the body. (c) Cercariae, young trematodes with oval body and a long tail. At the anterior end of the body is the anterior sucker at the bottom of which is the mouth. The mouth opens into the muscular pharynx, and this in turn into a bifurcated (Y-shaped) intestine. There is no anus. In the middle of the ventral side is the ventral sucker. How are the suckers used? How does the cercaria swim? Are there any cilia on the surface of the body? If possible observe the cercaria in the process of encystment. What becomes of the tail? How is the cyst wall formed? (d) Encysted worm: observe the little worms inside the cyst wall. (e) Mature sexual forms (worms which have come out from the cyst and lived for some time in the final vertebrate host). In mounted stained specimens observe: the shape of the body as compared with that of the cercaria, the posterior sucker (acetabulum), and the sexual organs,—the testes two in number, one on either side of the body about half way from the anterior to the posterior ends, the single ovary in the median line some distance posterior to the testes, the small lobulated shell gland near the ovary, and the large vitellaria, one on either side, lying partly above and partly below the intestine. The main trunks of the water vascular system will be seen along the sides of the body lying in folds beside the intestine. The excretory bladder is situated at the posterior ends of the body. It receives the posterior ends of the two lateral excretory trunks and opens to the exterior on the dorsal side of the body.

Make drawings of all the stages observed.

VIII. CROSSOBOTHRIUM OR TAENIA SP.

(A Tapeworm).

1. Examine an adult worm and notice that the body is flat and jointed.

2. Study a mounted specimen and observe: the scolex or head, its shape, size, the number and position of the suckers, the terminal beak or rostellum provided with hooks. Are there any sense organs on the scolex? The proglottides begin by faint constrictions just posterior to the scolex. Observe how they increase in size toward the posterior end of the worm. Along the sides of the body running through the whole series of proglottides, observe a clear tube the main trunk of the water vascular system. Can you find any connections between the trunks on the two sides? Does this trunk reach the scolex? How does it end anteriorly and posteriorly? Can you find any tubules emptying into the main tube? The sexual opening is found near the middle of each proglottis and in this worm (Taenia) on both right and left sides. Trace the ducts which lead from this opening toward the center of the body, and in the older proglottides, notice the uterus, centrally placed and filled with eggs. Each egg consists of a germ cell surrounded by yolk cells and all enclosed in a cell membrane. Only in a very favorable specimen can the glands which form each of these three constituents be seen. Observe the vagina or tube leading from the uterus to the sexual opening. The testes are rounded bodies on each side of the uterus connected by fine tubules which convey the spermatozoa to the *vas deferens*, and thence to the exterior. Study all of the above structures in prepared sections and observe in addition the body wall.

Make drawings of the entire worm, and of a single ripe proglottis to show all of the organs observed.

IX. ANGUILLULA ACETI (A Nematode Worm).

Place a drop of vinegar containing vinegar eels on a slide, cover and examine with the low power of the microscope. Observe:—

1. Form; Fusiform, anterior end blunter than posterior, body round in cross-section, unsegmented. Four longitudinal lines may be seen on the surface of the body, one dorsal, one ventral, and two lateral. Size; Female about 2 mm. long; male about 1 mm.

2. Cuticle; smooth, forming a thick coat over whole body.

3. Alimentary canal; mouth terminal in front; anus on ventral side in front of tip of tail; mouth cavity small; oesophagus 1/9 the length of body in female, 1/7 in male; intestine, a straight tube running from oesophagus to anus.

4. Reproductive system; this consists of long, blind tubes which lie in the body cavity, and open to the exterior behind the middle of the body in the female, and through the anus in the male; in the

latter the sexual opening is marked by two long slender spicules, which may be protruded or retracted.

5. Nervous system, is difficult to see. It consists of a circum-oesophageal ring from which a number of branches run forward, and two principle trunks run backward in the mid-dorsal and ventral lines.

6. Observe the manner of swimming. Run 50 per cent acetic acid and then 50 per cent alcohol under the cover glass. How does it affect the animals? Test in same way any other small animal. How do you explain the results?

X. LUMBRICUS TERRESTRIS (The Earthworm).

A. EXTERNAL CHARACTERS.

Place a preserved worm in a dissecting dish, cover with water, and observe:—

1. General form, color, irridescence.

2. Anterior and posterior ends? How do they differ? Dorsal and ventral sides; how distinguished? Right and left sides; are they symmetrical?

3. Body divided into metameres, or somites by grooves around it. Count the somites.

4. Between the 29th and 35th somites, a swollen light-colored region, the *clitellum*. How many somites does it cover?

5. The setae, stiff light-colored spines projecting from the surface of each somite, and easily felt with the fingers. How many are there on each somite, and how are they arranged? Do they all point in the same direction? Remove a seta, mount it in a drop of water, and examine it under the compound microscope. What is its general shape? Do its ends differ?

6. The cuticle. Soak an alcoholic specimen in water for a few minutes, and then strip off some of the cuticle. What is its color? Texture? Examine some from the ventral surface and note the cuticular sacs in which the setae are imbedded. What is their shape? Arrangement? Examine the cuticle under the high power and observe the striae crossing one another at right angles. At some of the intersections are pores to allow the escape of secretions of the epidermis.

7. Apertures. (a) The mouth, in front of the first somite, and below a protuberant lobe, the prostomium, which runs across the first somite on its dorsal surface. (b) The anus, a vertical slit at the end of the last somite. The following apertures are not easily

seen, and must be looked for with a hand-lens, or a dissecting microscope. They can often be seen by drying the surface of the worm, and then gently squeezing it, when a small drop will come out of the openings. (c) Sexual apertures. (1) Openings of spermaducts, or vasa deferentia; two openings surrounded by swollen areas on the ventral surface of the 15th somite. From these openings, grooves are often found passing back to the clitellum. (2) Openings of oviducts; two small pores on ventral surface of the 14th somite. (3) Openings of the seminal receptacles or spermatheca, two openings on each side between the 9th and 10th, and 10th and 11th somites, in line with the outer row of setae and posterior to them. (d) Nephropores; openings of the segmental organs or nephridia; two openings in each somite, one on each side, just dorsal to the ventral pair of setae. (e) Dorsal pores; openings into the body cavity at the anterior end of each somite on the median dorsal line.

Draw the anterior and posterior portions of the body to illustrate all that you have observed.

B. INTERNAL ANATOMY.

Extend the worm, ventral side down, in a dissecting pan, and fasten firmly by a pin at each end (the anterior one through the prostomium only); cover with water, and cut open carefully with fine scissors, making the incision along the dorsal side a little to one side of the dorsal median line. Do not cut deep, but merely through the body wall. Carefully cut through the partitions or septa along each side, stretch out the body wall to right and left, and fasten with pins.

Observe the following structures, dissecting as little as possible to make them out:

I. General Features.

1. Body wall, thick and firm and composed of three layers: (a) A thin cuticle on the outside; (b) a more or less colored layer, the epidermis; (c) a light-colored, and much thicker layer internal to the epidermis, the muscular layer.

2. Body cavity or coelom, with the digestive cavity passing through it from mouth to anus, and septa or transverse partitions dividing it into as many chambers as there are somites. Each septum passes from the digestive tract to the body wall. What is the relation of the septa to the external grooves?

3. Seminal vesicles, large lobed bodies between the 10th and 15th somites, partly covering the digestive tract.

4. Dorsal or supra-intestinal blood vessel, generally full of blood, and seen on top of the digestive tract, along the dorsal median line. In the 7th to 11th somites it gives off laterally 5 large pulsatile vessels, or "hearts", which pass around to the ventral side of the digestive tract.

5. Nephridia; or segmental organs, light-colored fluffy bodies attached to the posterior side of the septum, right and left, in each somite.

Make a sketch to show the above organs in place.

II. Digestive Tract.

Make out the following parts in the order named.

1. Pharynx, thick and muscular, extending back into the 6th somite and attached to the body wall by many radiating muscles.

2. Oesophagus, the narrow portion from the 6th to the 14th somites. On its sides in the 11th and 12th somites are 3 pairs of light colored swellings, the calciferous glands. Place one of the glands in a watch glass of dilute hydrochloric acid; explain results.

3. Crop. A large thin walled expansion in the 15th and 16th somites.

4. Gizzard, immediately posterior to the crop in the 17th and 18th somites, and with thick muscular walls.

5. Stomach-intestine, extending from the gizzard to the anus. It expands in each somite, and is contracted by each septum. Along its dorsal surface is a dark colored body, the liver, or pancreas. Cut open the intestine along one side and note the large ridge on its dorsal internal surface, the typhlosole. Cut open the gizzard and the crop, and note the lining of these structures and the character of the food contained.

Make a sketch of the digestive tract, showing the above mentioned structures.

III. Vascular System.

The dorsal blood vessel and the "hearts" have been mentioned. To observe the other principal vessels, remove the crop, gizzard, and oesophagus. Cut the oesophagus away from the pharynx, pull it gently back while cutting the septa which hold it is position, and leaving all the other organs in place. This will lay bare the white nerve cord on the median ventral line of the body cavity. Upon it the supra-neural blood vessel may be seen. In removing these parts of the digestive tract, the sub-intestinal vessel may be seen on its ventral side.

IV. Reproductive System.

1. Seminal vesicles; composed of 3 pairs of white sacs arising from a median portion below the oesophagus. This median portion is subdivided into an anterior and a posterior part.

2. Seminal receptacles; 2 light colored sacs on the ventral surface of the body-wall, on each side of the median line and attached to septa between the 9th and 10th and 10th and 11th somites.

3. Ovaries; very small light colored bodies with pointed tips and rounded bases on the anterior wall of the 13th somite, not very far from the middle of the ventral surface, one on each side, right and left.

4. Oviducts; these are also not easily seen, but form what appear as thickenings of the wall between 13th and 14th somites.

5. Cut off the lateral lobe of a seminal vesicle, cut open its median part and carefully wash out its soft contents to show the following structures; great care in dissection and observation is necessary. (a) Vasa Efferentia; large folded or convoluted masses which form the funnel-like openings, one on each side of the median line in the 10th and 11th somites. From these, delicate thread-like ducts pass back on each side to unite in somite 12 to form the Vas Deferens, which passes along the body wall one on each side of the median line, as far back as somite 15 where it opens to the exterior. (b) Testes; four small white bodies, a pair in each somite, inside the seminal vesicles in part concealed by the funnels of the vasa efferentia, and attached to the posterior surfaces of the septa between somites 9 and 10, and 10 and 11, two on the right and two on the left of the median line.

V. Nervous System.

1. The nerve cord; extending the whole length of body on the median ventral line, lying in the body cavity but near the body wall. In each somite it expands to form a ganglion and gives off three pairs of nerves. (a) Two large pairs arise from the ganglion. (b) One smaller pair arises from the slender part of the cord (connective) near the anterior end of the somite.

2. Circum-oesophageal nerve ring. Raise the oesophagus and trace the nerve cord anteriorly to its division into right and left halves which pass around the digestive tract to form a ring which unites with the brain on the dorsal side of the oesophagus.

3. Brain; connected as above shown with the ventral nerve cord,

but lying dorsal to the pharynx. Note the nerves given off from the brain and also from the connectives on the sides of the pharynx.

Make a diagram to show the reproductive and nervous systems.

VI. Body Wall.

Pin out part of the body wall quite flat and note that the muscular layer of the wall is interrupted along four longitudinal lines in which are the setae in sacs or setigerous glands; four of these occur in each somite. Between somites 12 and 13 some of these glands are conspicuously large; tease out one and note under the microscope the color, shape and hardness of the setae.

C. ANATOMY AND PHYSIOLOGY OF LIVING SPECIMENS.

I. Movements.

1. Place a worm upon moist filter paper and observe the direction and method of movements.

2. In small light-colored worms note the contraction of the dorsal blood vessel and the movements of the blood toward the anterior.

3. Gently touch different parts of the body and note which are the most sensitive.

4. Place the worm under a glass vessel with some cotton saturated with chloroform, the vapor of which will render the animal insensible; when motion has ceased remove the worm and cut it open as in the specimen previously dissected, but only in the anterior region and a little to one side of the median line. Keep the specimen wet with physiological salt solution.

II. Vascular System.

If the specimen is not quite dead observe:

a. The contraction of the hearts, dorsal vessel, and sub-neural vessel; in the latter the wave of contraction passes backward.

b. Small blood vessels passing from the dorsal vessel to the digestive tract, and from the ventral vessel to the body wall and to the septa.

c. Fine vessels seen upon the septa, body walls and the nephridia.

III. Coelomic Fluid.

Puncture the body where not yet opened and take out in a fine pipette some of the fluid of the body cavity, examine under a high power and note:—

1. White amoeboid corpuscles.

2. Yellow granules, from the cholorogogue cells (See D II. 1).

3. Bacteria or other foreign bodies, especially Gregarina, which are often present.

D. HISTOLOGY.

I. Body Wall.

Examine prepared transverse sections of the body; observe body wall, now seen to be made up of five layers:

1. Cuticle, a thin non-cellular layer (membrane) often torn off.

2. Deric epithelium or epidermis, a single layer of cells many of which are swollen (gland cells).

3. A thin outer layer of circular muscle fibres with blood vessels and connective tissue nuclei among them.

4. A thick layer of longitudinal muscle fibres or plates, arranged in elongated groups of eliptical form.

5. Peritoneum or coelomic epithelium, a thin layer of granular protoplasm containing nuclei, lining the body cavity.

Sketch a portion of the body wall to show the above parts.

II. Digestive Tract.

In its wall four layers are to be seen.

1. Chlorogogue cells, large and more or less elongated and irregular.

2. An outer layer of longitudinal muscle fibres cut across.

3. A layer of circular muscle fibres and of blood vessels (not easily made out).

4. Enteric epithelium; a single layer of elongated cells with stained nuclei and a thin cuticle over their central ends through which fine cilia project into the lumen of the gut.

Sketch a small part of the digestive tract to show the above.

III. Nervous System.

In a transverse section of the ventral cord, note:

1. An outer muscular sheath or coat.

2. Large ganglion cells in groups or clusters.

3. A mesh work of fine fibres.

4. Very large clear "giant fibres" each in a definite sheath.

5. In some of the sections the nerves are to be seen as they pass from ganglion cells to the body wall.

IV. Body Cavity.

Some of the sections will show the following structures:

1. The corpuscles of the coelomic fluid.

2. Blood vessels cut across and filled with coagulated blood.

3. Mesenteries or dorsal and ventral membranes connecting the digestive tract with the body wall on the median line.

4. Septa and nephridia cut at various angles.

Make drawings to show the histology of the nervous system and body cavity.

XI. CAMBARUS SP. (The Crayfish).

A. GENERAL CHARACTERS.

I. Body.

Note that the animal has a body proper and a series of paired appendages. The body is bilaterally symmetrical and divided into a posterior jointed *abdomen* and an anterior portion the *cephalothorax*. The entire body is covered by a hard calcarious shell the *exoskeleton* which is flexible at the joints where movement may take place.

II. Appendages.

Note that all of the *appendages* are jointed, that they are attached in pairs to the ventral surface of the body and that they vary much in size and form.

Make a drawing of the crayfish as seen from the dorsal side.

III. Apertures.

Make out the following *apertures* in the body wall:

1. The mouth seen under the anterior part of the cephalothorax after separating from one another the crowded appendages.

2. Anus, a much elongated slit upon the lower side of the terminal piece of the abdomen, the telson.

3. Genital openings on the basal joints of the legs: (a) In the male on the delicate papilla on the last appendage of the cephalothorax (one on the right and one on the left); (b) in the female an opening with a valve-like edge on the antepenultimate appendage of the cephalothorax (one on the right and one on the left).

4. Auditory organs: A small opening on the appendage (antennule) just under each eye stalk.

5. Green glands: A large opening on the first joint of the next following appendage (antenna) on each side.

B. ABDOMEN.

This is made up of six segments or somites bearing appendages and a terminal, seventh piece, the *telson*, which is subdivided

by a transverse hinge and bears the anus. Carefully examine the third abdominal somite. The following surfaces are found upon it: (a) *Tergum,* the dorsal arched portion overlapped anteriorly by the preceding tergum. (b) *Sternum,* the ventral portion between the appendages, composed of a transverse bar and a more calcified cuticle where movements take place in bending the abdomen. (c) *Pleuron,* the downward projecting portion on each side, overlapped in front by the pleuron of the preceeding segment. The appendages are attached to the body by soft flexible cuticular parts of the exoskeleton.

Each abdominal appendage consists of the following parts:

a. *Protopodite*: This is the proximal part of the appendage and is divided into a long joint, and a small ring-like piece by which it is attached. It bears distally two parts.

b. *Endopodite*: This is the part nearer the middle line.

c. *Exopodite:* The portion farther from the middle line.

C. CEPHALOTHORAX.

a. The large shield-like part of the exoskeleton covering the cephalothorax above and on the sides is the *carapace,* which is prolonged in front into the frontal spine or *rostrum.*

b. A groove, the *cervical suture* runs across the carapace and marks off the head from the thorax.

c. On the ventral side the region between the appendages is very narrow; the anterior appendages project forward and not downward as do the more posterior ones.

d. The *locomotor appendages* are attached to the thorax; the posterior pair are upon a movable somite while all the others arise from a fused single mass continuous with the head.

e. The free lateral part of the carapace, above the appendages, is the *branchiostegite.* Raise its edge and see that it covers the gills.

f. Respiratory organs: Remove one of the branchiostegites, study the gills under water and observe: Six of them are attached to the appendages, *prodobranchiae;* eleven of them are attached to the soft cuticle joining the appendages to the body *arthrobranchiae.* At the anterior end of the branchial cavity a canal leads forward toward the mouth and in this lies the flat part of the second maxilla called the *scaphognathite.*

D. APPENDAGES.

Carefully remove all of the appendages from one side *with all*

the basal parts of each, see which are alike and then *draw one of each kind or set,* keeping all of the small ones in water in watch glasses.

I. Abdominal Appendages or Swimmerets.

Composed of a two joined *protopodite*, and an *exopodite* and an *endopodite* each with many joints; found on all but the first and sixth somites (and the second also in the male) where the appendages are more or less modified.

II. Thoracic Appendages.

There are five pairs of ambulatory, and three pairs of masticatory appendages (the *maxillipedes*).

a. The posterior pairs of ambulatory appendages have the following seven joints: (1) *Coxopodite,* the short and very thick basal joint. (2) *Basipodite,* a very small and conical joint. (3) *Ischiopodite,* cylindrical and with a groove around it. (4) *Meropodite,* very much longer than the last. (5) *Carpopodite*, about half as long as the last. (6) *Propodite,* slender and long. (7) *Dactylopodite,* the short, pointed terminal piece. (Of these (1) and (2) probably correspond to the protopodite of the abdominal appendages, and the other five to the endopodite, as may be seen by comparing all of the other appendages with the third maxilliped.

b. The next pair of appendages have in addition a *branchia* and *epipodite* upon the coxopodite and extending up into the branchial chamber.

c. The third and fourth appendages (counting forward) differ in having the propodite produced opposite the dactylopodite to form a pair of forceps.

d. In the large anterior pair of ambulatory appendages, the *chelae,* the forceps is greatly enlarged and the basipodite and ischiopodite are united into one piece.

e. The third or posterior maxilliped should be carefully studied. Note: (1) The large basal part, *protopodite*, bears a long five jointed *endopodite* and a slender many jointed external *exopodite*, besides a curved lamella, *epipodite,* lying in the branchial chamber and bearing a branchia. (2) The protopodites and endopodites make up together a seven jointed organ like the ambulatory appendages.

f. The second maxilliped differs from the last mentioned, chiefly in the size of the endopodite.

g. In the first maxilliped the endopodite is short and flat, the protopodite two jointed and foliaceous, the epipodite has no gill.

III. Cephalic Appendages.

These are the maxillæ, mandibles and antennæ.

(a) Second or post maxillæ; the endopodite is not jointed, while the two parts of the protopodite are subdivided or cleft; the large oval plate, *scaphognathite*, acting to bail water out of the branchial chamber, represents the epipodite and probably also the exopodite. (b) First maxilla; this is very small and lies close to the mandible. It is divided into three parts representing the coxopodite, basipodite and endopodite. (c) Mandibles; each has a strong basal part bearing a two jointed *palp* or endopodite. (d) Post antenna (antenna proper); each has a two jointed protopodite with the opening of the green glands on a tubercle on the proximal joint, the scale-like plate is the exopodite and the long, filiform, many jointed part is the endopodite. (e) First antenna (antennula); here the protopodite has three joints and bears a long many jointed endopodite, and a similar exopodite, while upon its large proximal joint is the opening of the auditory organ, surrounded by hairs.

Compare your drawings of the different kinds of appendages, labelling similar parts with the same name; the 19 pairs may be regarded as modifications of such a one as the third maxillipede.

E. INTERNAL ORGANS.

Pin the crayfish down under water, dorsal side up, and carefully remove the carapace bit by bit with strong forceps, commencing at the free posterior border.

I. Heart.

Posterior to the cervical suture, a median chamber is laid bare, the pericardial sinus, within which lies the polygonal, flat heart which has six openings into the pericardinal sinus, two on the dorsal surface, two on the lateral surfaces, and two on the ventral surface.

II. Reproductive Organs.

Carefully remove the heart to expose the reproductive organs. (a) Testes; in the male, these form a Y-shaped mass with the smallest of the three lobes passing back along the median line. (b) Vas deferens. Cut away the thoracic wall on one side and trace the much convoluted tube from the union of the posterior and anterior lobes of the testes down to the external genital opening on the posterior ambulatory appendage on that side. (c) Ovary. In a female specimen the larger reddish ovaries have

the same general form and position as the testes in the male. (d) Oviducts. These are short and go directly down from the ovary to the openings on the third, or middle, ambulatory appendages.

Make a drawing of your dissection, showing all these organs in place.

III. Digestive Tract.

(a) Carefully remove the anterior part of the carapace and notice the very large sac-like stomach anterior to the heart. Pass a probe into it through the mouth and short oesophagus. (b) Dissect away the exoskeleton and muscles and follow the intestine from the stomach to the anus. Immediately posterior to the stomach is the "mid gut" having a short dorsal diverticulum on it. (The remainder of the intestine is short and simple.) (c) The digestive gland (the so-called "liver") forms a yellow mass opening by a duct on each side of the mid gut. Wash away its contents if the duct cannot otherwise be found. (d) Remove the intestine and cut open the stomach along one side (under water) and note a large round (cardiac), and a narrow posterior (pyloric) portion. The chitinous lining forms in the cardiac portion three conspicuous tooth-like thickenings. In the pyloric region ridges, set with hairs, reduce the lumen of the stomach to a narrow slit.

Draw a side view of the digestive tract.

IV. Nervous System.

Remove the muscles of the abdomen until the nerve cord is seen along the ventral wall of the body. (a) Note the relation of the ganglionic swellings to the somites. (b) Follow the cord into the thorax; here it enters a canal, the roof of which must be broken off bit by bit with forceps to show the nerve cord. Note the number of ganglia in the thorax. (c) The cord divides at the oesophagus into a right and left half which meet again at the brain. The brain, or supraoesophageal ganglion, lies just posterior to the eye stalks, close to the exoskeleton, and sends a large nerve into each of the eye stalks.

Make a drawing of the nervous system.

XII. VENUS MERCENARIA. (A Pelecypod Mollusk).

I. Physiology.

If living speciments are available, allow powdered carmine to settle slowly past the openings of the siphons and determine the direction of the current of water for each. Observe if possible the method of

locomotion. Touch portions of the animal and find what parts are the most sensitive.

II. Shell; Exterior.

Note its general shape, and that it is composed of two symmetrical parts, the valves. For each valve notice:

(1) The outline.

(2) A swelling, the *umbo,* ending in a point, the *beak,* from which growth has proceeded.

(3) The lines of growth. Were the valves cut off along one of these lines, the shape would not be changed. Why are the lines arranged in this manner? How were they formed? The two valves are joined by the ligament. The margin bearing the ligament is dorsal, and that toward which the beak points is anterior. Which valve is right and which is left?

Draw a valve showing the points observed.

Pry the two valves apart and insert a knife blade between the mantle and one valve of the shell. Notice that the lobes of the mantle are loosely attached to the shell at their margins, and more firmly attached at a point a half inch or more back from the margin.

III. Shell; Interior.

Separate the mantle from one valve and cut the *adductor muscles* where they are attached to this valve. Why do the valves gape now? Press them together and notice that they stay closed only when held. Remove a valve and study its interior.

(1) Find the large scars where the anterior and posterior *adductor muscles* were attached.

2. Find smaller scars where the anterior and posterior *foot muscles* were attached. The anterior scar is dorsal and a little posterior to the corresponding adductor muscle scar. The posterior scar connects with the dorsal portion of the corresponding adductor muscle scar.

3. The ventral borders of the adductor muscle scars are connected by a distinct line, the *palial line.* What forms it? The posterior end of this line is indented to form the *palial sinus.* What is the meaning of this sinus?

4. Along the dorsal margin of the valve notice prominences, the *teeth.* There are two kinds of teeth. The anterior,*cardinal,* consist of short elevations. The posterior, *lateral,* are not very prominent, but extend for some distance along the dorsal margin.

Notice that the teeth on the two valves interlock. What is their function?

Draw a valve as seen from the inside.

5. By examining a shell of Mytilus, or Unio, near its margin, the typical three layers of which it is composed can be seen. How is it possible for all three layers to be secreted by the mantle, which lines the inside of the shell? Can you find any reason for more than one layer?

IV. Mantle.

This consists of two lobes (one of which is normally applied to the inner surface of each valve of the shell), that are united dorally.

1. The free border of each lobe is thickened and contains muscles which are attached along the palial line. What function do these muscles perform?

2. The posterior portions of the lobes of the mantle are thickened and united to each other so as to form two tubes (in Unio the ventral tube is formed by contact only), the *siphons,* through which water passes into and out of the shell.

3. See how the muscles of the siphons are arranged and attached. Does the attachment bear any relation to the palial sinus?

V. Visceral Mass and Foot.

These portions form the large median mass. The viscera are contained in the dorsal portion. The ventral portion is hard and muscular and forms the *foot.*

VI. Gills.

These consist of two thin, striated lamellae (gills) on each side of the foot. The inner and outer gills of each side are attached to each other along their dorsal borders, and the outer gill is also attached to the mantle; the inner one is attached to the visceral mass anteriorly, while posteriorly the inner gills of the two sides are united, thus partially separating an upper cavity, the cloacal chamber, from a lower one, the branchial chamber. These attachments occur only at the dorsal borders of the gills, which otherwise hang free in the branchial chamber. The ventral siphon opens into the branchial chamber, while the dorsal siphon opens out from the cloacal chamber. Labial Palps: A pair of small triangular flaps on each side of the visceral mass. The outer palps are united above the mouth, which is situated just posterior to the ventral

border of the anterior adductor muscle, to form a kind of upper lip. The inner palps unite to form a fold corresponding in position to a lower lip.

Make a drawing showing the arrangement of the soft parts.

Cut off a bit of a gill with scissors and examine it in sea water under a low power of the microscope. Observe:

a. The surface is marked by a series of parallel ridges, the gill filaments, which are united together, side by side, by interfilamentar junctions, to form a lamella; the spaces between the interfilamentar junctions are the ostia. Each gill is composed of two such lamellae, which are united together at intervals by interlamellar junctions; the spaces between these latter junctions form water tubes, which open dorsally into the cloacal chamber. With a high power observe:

b. The movements of the cilia which cover the filaments. Place a little powdered carmine on the surface of a gill submerged in sea water and see what happens.

Draw a surface view of a portion of a gill as seen under the low power.

VII. Circulatory System.

Anterior to the posterior adductor muscle on the dorsal side is a clear space, the pericardium, in which the heart lies. Open the pericardium and observe the beating of the heart. The heart consists of three parts:

1. A central ventricle, which surrounds the intestine and gives rise to a blood vessel at each end.

2. Two triangular auricles, which receive blood from the gills and open into the sides of the ventricle.

Draw the heart.

VIII. Excretory and Genital Systems.

Beneath the pericardium is a pair of dark colored excretory organs. Each communicates with the pericardium by a small opening and with the cloacal chamber by another small opening. The genital glands are light colored organs that, during the breeding season, extend through the principal part of the visceral mass.

IX. Nervous System.

1. Cerebral ganglia; carefully remove the body-wall by the side of the oesophagus and observe a rounded, slightly yellow organ, about the size of a pin-head, lying just posterior to the dorsal

border of the anterior adductor muscle. The ganglia of the two sides are united by a commisure which passes anterior to the oesophagus.

2. The Visceral ganglia lie on the ventral side of the posterior adductor muscle and may be exposed by separating the united lamellae of the inner gills. The two are connected by a short commissure with each other and are connected with the cerebral ganglia by connectives that may be traced forward a short distance without dissection. A large nerve leaves the posterior end of each ganglion and supplies the posterior margin of the mantle and the siphons.

3. The Pedal ganglia lie in the substance of the foot, dorsal to the muscular part and in front of a loop of the intestine. With a sharp scalpel make a median section of the foot extending it some distance into the visceral mass; this will expose these ganglia. They are connected together by a broad commissure, and with the cerebral ganglia by connectives.

Draw the nervous system, showing the ganglia, connectives and commissures.

X. Digestive System.

1. The mouth lies ventral to the anterior adductor muscle. Is it provided with teeth or any organs of prehension? What is the nature of the food and how is it brought to the mouth?

2. The oesophagus leads from the mouth to the stomach, which is an enlarged portion of the digestive tract surrounded by a brownish gland commonly called the "liver".

3. Following the stomach is a much folded intestine, with walls so thin that it is not practicable to trace it in detail. The hinder portion of the intestine runs through the heart and opens by the anus into the cloacal chamber, ventral to the posterior adductor muscle. The general arrangement of the digestive system is well shown by a median sagittal section of a preserved specimen.

Draw the alimentary canal.

XIII. ASTERIAS VULGARIS. (The Starfish).

A. EXTERNAL CHARACTERS.

Examine a specimen and note that:

1. It consists of radiating arms and a central disk.

2. That the surface by which the animal clings, the oral surface, is different from the other, aboral surface, and that both surfaces are covered with short spines.

3. On the aboral surface of the disc, near the junction of two of the arms is a small, conspicuously colored, circular body, the *madreporic plate*. The two arms adjacent to this plate are sometimes referred to as the "bivium" and the remaining three as the "trivium". The radial symmetry of the animal is disturbed externally only by the madreporic plate. Examine this plate with a lens and determine its structure.

4. On the oral surface is the mouth. Note its size and see if it is provided with jaws of any sort.

5. Radiating from the mouth are the *ambulacral grooves,* one on each arm. In these grooves are the ambulacral, or tube feet. Do they have a definite arrangement? Along the sides of the grooves are slender spines which differ from those covering the general body in being movable.

6. Scrape the tube feet from a portion of an ambulacral groove of a dried specimen and notice the pores through which the feet pass to organs within the arm. Notice also the exposed ambulacral plates, and determine their relation to the pores.

Draw figures of the oral and aboral surfaces of a starfish and a diagram to show the relation of the ambulacral plates and pores.

7. On the aboral surface of the body find the dermal branchiae, folds of the soft part of the body-covering, extending out between the calacarious plates, which give the body its rigidity. Look with a hand lens for small two-jawed structures around the bases of the spines. Remove some of these *pedicellaria* and examine under the microscope.

Draw a pedicellarium.

B. INTERNAL STRUCTURE.

Make the dissection under water and in cutting through the integument be careful not to injure the underlying soft parts.

With scissors cut through the aboral wall near the tips of the rays of the trivium. Carry the cuts along the sides of the rays to the disk. Lift up the integument at the tip of each arm and carefully cut away the mesenteries which attach the organs to it. Cut the membranes which extend into the disk opposite the junction of the arms, and remove the three rayed flap of integument thus freed, cutting as close as possible to the madreporite, but leaving this in place.

I. Digestive System.

In studying this system you should constantly bear in mind the

peculiar method by which the animal feeds, as the digestive system is highly modified to suit this method.

1. The short cone-shaped intestine and the intestinal caeca were probably removed with the integument. The intestine probably does not function and may be regarded as a vestige. It opens near the center of the disc, on the aboral side, by a very minute anus which is hard to see.

2. The stomach, which occupies the greater part of the space within the disc, is composed of a small aboral portion, the *pyloric division,* that receives the ducts from the hepatic caeca, and a larger lobed, cardiac division, into which the mouth opens. The cardiac portion may be everted through the mouth thus being turned wrong side out. Five pairs of muscles, which draw this portion of the stomach back into place, may be seen attached to the ridges formed by the ambulacral plates in each arm.

3. In each arm is a pair of long, glandular organs, the hepatic caeca. The ducts from each pair unite and join the pyloric division of the stomach by a common duct. These are digestive glands. What reason is there for ten enormous digestive glands? Does this have anything to do with the method of feeding?

Make a drawing of the digestive system of the disc and of one arm.

II. Reproductive System.

Turn the hepatic caeca to one side and note the ovaries or testes. The sexes are separate, but the gonads have the same general appearance in both sexes. They vary in size according to the season of the year, sometimes being so small that they are not easily found, and again being nearly or quite as large as the hepatic caeca. With a pair of forceps lift up one of these organs and see where it is attached. It is at this point that the reproductive cells reach the exterior. How many gonads are there?

Draw the gonads in one arm of your figure.

IV. Water-Vascular System.

1. Carefully remove the side of the stomach next to the bivium, being very careful not to disturb the stone canal, which runs from the madreporic plate to the margin of the membrane about the mouth. By the side of the stone canal is a thin band of tissue formerly supposed to be a heart. It is now generally believed to be connected with the reproductive system, and is commonly referred to as the axial organ. It has nothing to do with the system now under consideration.

2. The *circulár canal,* which is joined by the stone canal at the outer margin of the peristomal membrane, follows the margin of the membrane and thus encircles the mouth. Originating from it at points very near the ampullae of the first tube feet are nine small vesicles, *Tiedemann bodies.* They are smaller than the ampullae and project toward the mouth. The position where the tenth Tiedemann body might be expected is taken by the stone canal.

3. Leaving the circular canal are five *radial water tubes,* one for each arm. These tubes lie along the oral surfaces of the ambulacral plates, and are accordingly not visible on the inside of the animal. The position of the tube can be best understood by making a transverse section of the arm. It will then be seen either in injected or uninjected specimens, lying immediately below the ambulacral plates. In injected specimens it may be followed by dissecting on the oral side, from the circular canal to the extremity of the arm where it ends in a small tentacle.

4. Along the sides of the ambulacral ridges, within the body cavity, are rows of little bag-like ampullae. Determine their relation to the ambulacral pores. In a dissection it is hard to find the connecting tubes which join the radial tubes to the tube feet, but they can sometimes be seen in sections of the arms of injected specimens. They can readily be seen in microscopical preparations.

The water vascular system is very distinctive for the *echinoderms,* and you should understand perfectly: (1) How the tube feet are extended. (2) What causes them to adhere. (3) The connection between tube feet, ampullae, connecting canals, radial water tubes, circular canal, stone canal and madreporite. (4) How it is possible to extend one foot without extending others.

Make a drawing showing the arrangement of the water vascular system.

IV. Nervous System.

This is not easily studied by dissection. It consists of a nerve ring which encircles the mouth, and lies just ventral to the circular water canal, and five radial nerves that extend down the arms just beneath the radial water tubes, to end at the tips of the arms in pigment spots. The whole central nervous system is superficial and forms a portion of the outer covering of the body. The radial nerves can be seen by separating the rows of ambulacral feet, but it is much more satisfactory to study them in prepared sections.

C. PREPARED SECTIONS.

Study prepared sections of the arm of a starfish and observe:

1. The hepatic caeca. How are they supported? What is their structure?

2. The radial canal, connecting tubes, tube feet and ampullae.

3. The thickened, deeply staining nerve, between the tube feet and below the radial water tube.

4. The perihaemal canal, divided by a thin partition, and lying between the radial water tube and the radial nerve.

Make a drawing of a section of an arm which will show these points.

D. PHYSIOLOGY.

a. Observe a living specimen in the Vivarium and Note: (1) Method of Movement. (2) Method of Feeding. How does it open an oyster? How does it swallow the oyster?

b. Place a starfish in a dish of sea water and observe its reactions to the following stimuli:

(1) Mechanical; touch the end of an arm with your pencil and note the response.

(2) Gravity; place the animal on its aboral surface and observe how it rights itself.

(3) Place the animal so that bright sunlight falls on the tip of an arm and note the response.

RANA SP. (The Frog).

A. GENERAL STRUCTURE.

I. External Characters.

Note the smooth moist skin over the entire animal; the absence of exoskeleton; the head, trunk, two pairs of limbs; the absence of a tail and of a neck.

a The head. Observe:

1. The eyes are prominent and have lids; the ears are covered over by a modified part of the skin, *membrana tympani,* posterior to the eyes; the two *anterior nares,* or nostrils; the position of the mouth opening; the soft flexible throat and hard parts of the endoskeleton felt on the dorsal side of the head.

2. Pass a bristle far into the anterior nares and one into the ear through a hole cut in the *membrana tympani;* on opening the mouth the bristles will indicate its communications with the nostrils

and tympanic cavity. The second bristle appears in the *Eustachian recess* at the side of the posterior part of the mouth. In the male a small opening anterior to this recess leads into the *buccal sac* which can be distended, by means of a small blow-pipe. Turn the fleshy tongue forward and notice its mode of attachment. Note the slit of the *glottis* and the posterior opening of the mouth into the *oesophagus;* pass a bristle into the former and a large probe into the latter. There are thus two median and six paired openings. from the mouth cavity in the male frog. Note the small teeth.

b. The trunk.

This tapers towards the posterior end where the *cloacal aperture* is seen near the dorsal surface. Beneath the skin the hard endoskeleton can be felt on the dorsal side and on the anterior part of the ventral side.

c. The limbs.

1. The anterior pair divided each into three regions, *brachium, antebrachium, manus;* the latter with four *digits,* the innermost of which bears a swollen cushion in the male.

2. The much longer posterior pair each divided into three regions, *femur, crus, pes,* the latter with five long digits connected by a *web.* There is a large firm prominence on the inner side of the ankle; *callosities* are found under the joints of both *pes* and *manus.*

II. Internal Characters.

a. Place the frog under a bell-jar with a sponge saturated with chloroform; when dead pin out under water on its back.

b. Cut through the skin along the median ventral line from the posterior end to the jaw (raising the skin from the body and not cutting deep); cut transversely at each end of first cut and turn aside the two large flaps thus made.

c. On the flap of skin on each side is seen a large vein near the shoulder, the *musculo-cutaneous vein.* The muscular walls of the abdomen are covered by a thin, shining connective tissue sheath, *aponeurosis,* through which in the median region is seen the *rachis abdominis* passing from the pelvis to the sternum and somewhat divided by transverse lines into segments or *myotomes.* Through this muscle is seen the dark blood of the *anterior abdominal vein* on the median line.

d. With a pair of forceps raise the body wall and carefully cut it through by a slit to the right of the median line; continue this cut from pelvis to sternum and make transverse cuts as in the

skin so as to throw back a flap of body wall on each side; the left one should show the anterior abdominal vein on its exposed surface.

e. With forceps raise the sternum and carefully cut off the fibrous bands seen passing to soft organs dorsal to it; with strong scissors cut through the sternum and other hard parts on the median line carefully holding it up away from the soft parts dorsal to it. Turn each half outward and pin firmly; pin the anterior limbs out at full length.

d. The *liver* is conspicuous, forming a large brown mass with the pericardial sac just anterior to it.

f. Carefully cut away the membranous pericardium to expose the heart; then with great care clean off bit by bit the tissue covering the vessels at the anterior end of the heart.

g. (1) Note the firm conical posterior portion of the heart, the *ventricle*. (2) The cylindrical *truncus arteriosus* arises from the right side of the base or anterior end of the ventricle and passes obliquely forward to divide into two large branches. (3) The *atrium* forms a thin walled sac dorsal to the truncus and anterior to the ventricle (it is divided *internally* into two auricles). (4) The *sinus venosus* can be seen by carefully raising the ventricle to one side; it forms a thin sac dorsal to the ventricle and atrium and receives three large veins (two anterior or *superior venae cavae* and one large posterior or *inferior vena cava.*) Two *pulmonary* veins open into the left auricle by a single opening. (5) Each branch of the truncus divides into three arteries, the most anterior is the *carotid*, the most posterior the *plumino-cutaneous* and the middle one the *systemic aortic arch* which unites with its fellow dorsal to the heart to form the *dorsal aorta.*

Make a diagram of the heart and vascular trunks.

h. Pulsations of the heart. Observe: (1) A regular sequence of contraction and dilation. (2) The atrium contracts, then the ventricle, and immediately after the truncus. (3) On raising the ventricle, the sinus venosus can be seen to contract before the atrium. The contraction proceeds in the same order as that followed by the blood in passing through the heart.

i. Anterior to the heart note the broad flat transverse *mylohyoid* muscle through which can be seen the long first vertebral nerve or hypoglossal. Note also the hard portuberant *larynx* and on each side of this a small soft body, the *thyroid gland.*

j. Posterior to the heart, note the following viscera: (1) *The*

liver with its larger left lobe divided into two parts; on raising the posterior border, the *gall bladder* is seen as a greenish sac on the right side; also the *hepatic-portal* vein, which enters the left lobe of the liver. *The stomach,* an elongated white body on the left side under the posterior edge of the liver. (3) A convoluted tube, *the intestine* passing from the stomach to the right and then posteriorly to finally enter the pelvic cavity as an expanded rectum. It is slung by a delicate membranous fold of peritoneum, the *mesentery,* which is full of blood vessels. (4) The *fat masses,* long slender yellow masses on each side in the dorsal part of the body cavity anterior to the reproductive glands. (5) The *urinary bladder,* a large bilobed sac ventral to the rectum (it can be inflated through the cloaca by means of a blow-pipe).

k. Cut off all the dorsal part of the liver with strong scissors, cut open the body wall in the pelvic region without injuring the rectum. (1) The *cloaca* is now exposed; a bristle may be run from it into the rectum. (2) Uncoil the intestine and fasten to one side to expose the *spleen* (a small red body near dorsal part of mesentery). (3) The *pancreas* is also seen as a pale-colored compact mass in the mesentery between the stomach, liver and small intestine. The bile duct from the gall bladder passes through the pancreas to open into the small intestine. (4) The oesophagus is a short straight tube; pass a probe from the mouth into the stomach.

Make a drawing of your dissection to show all of these parts.

l. Remove the stomach, liver, mesentery and organs connected with it. (1) Posterior to the fat masses, lie the *reproductive glands,* in the male yellow, rounded *testes;* in the female, folded or lobed, yellow *ovaries* (when the eggs are nearly ready for laying, each is a large sphere, light on one side and dark on the other, and the lobes of the ovary are so distended by great masses of ova as to fill most of the body cavity). (2) Sexual ducts: in the male, each testis sends numerous small thread-like ducts, *vasa-efferentia,* into the kidney lying just posterior and dorsal to it. In the female the *oviduct* is a long convoluted tube opening into the cloaca posteriorly and passing forward on each side to open by a funnel into the body cavity near the oesophagus. It has no connection with the ovary. (3) The kidneys are elongated, red masses close to the vertebral column; on the ventral surface of each is an elongated yellowish body the *adrenal body.* Entering the kidneys on their external side are the *renal-portal veins;* leaving the kidneys on their mesial side are the branches of the *inferior vena cava.* (4) Ureter, a whitish

duct on each side passing from the outer side of the kidney into the cloaca. (In the male this serves also as a vas deferens.)

Make a diagram of the urino-genital system of your specimen.

m. Tag your specimen with your name and put it into a jar of preserving fluid until the next exercise.

III. The Nervous System. (Preserved Specimen.)

a. Cut the skin along the median dorsal line and reflect it. Remove the muscles from the vertebrae. Open the neural canal by cutting into the membrane just posterior to the skull, and bit by bit, pick off the roof of the brain cavity with strong forceps or scissors. Remove the dorsal part of the vertebral arches in the same way. A delicate pigmented membrane (*pia mater*) covers the brain and the spinal cord but may be concealed in the latter region by soft tissue (coagulation products after death) that can be washed away with a stream of water from a pipette.

b. *The Brain;* if this is not injured in exposing it, note:

1. *Rhinencephalon* or anterior part made up of two *olfactory lobes* extending anteriorly from a common median part as two cylindrical so-called *olfactory nerves* to branch inside the nasal chambers.

2. *Prosencephalon* composed of two large masses, the *cerebral hemispheres*, separated by a median groove.

3. *Thalamencephalon*, a mass between and posterior to the hemispheres. Upon it is a very small *pineal gland* and below this a central cavity, the third ventricle, bounded on the sides by masses called *optic thalami.*

4. *Mesencephalon*, a pair of large, rounded, hollow bodies, the *optic lobes.*

5. *Metencephalon* or *cerebellum*, a small mass extending across the anterior edge of a large triangular cavity, the *fourth ventricle.*

6. *Myelencephalon* or *medulla oblongata*, forms the remainder of the brain posteriorly and contains the fourth ventricle covered over by a vascular part of the *pia mater.*

c. *Spinal Cord or Myelon.*

1. Forming the continuation of the medulla oblongata outside the skull it rapidly tapers at about the fifth or sixth vertebra to form a slender filament. On its dorsal surface is a median line, the dorsal fissure.

2. On each side ten *spinal nerves* arise from the cord. By carefully raising the cord a little, towards the posterior end, and

searching with a pocket lens, the nerves are seen to arise each by two roots, one dorsal, one ventral.

Make a sketch of the central nervous system as thus exposed.

d. Cut the olfactory nerves away from the skull, gently turn the brain back cutting all the nerves close to the skull and thus remove the brain (as entire as possible), and part of the spinal cord. Place in a dish of water and study the ventral side with a pocket lens.

1. *Optic chiasm* or *commissure,* a transverse elevation at the posterior end of the cerebral hemispheres continued up on the sides of the brain towards the optic lobes as the *optic tracts,* and giving rise in the other direction to the optic nerves, (cut off in removing the brain).

2. Tuber cinereum: a rounded somewhat two-lobed elevation posterior to the chiasm, continued ventrally into the conical *infundibulum,* or slender neck bearing a small conical mass, the *pituitary body* or *hypophysis cerebri.*

3. *Crura cerebri*: the anterior continuation of the medulla as the large nerve mass dorsal to the above parts, extending anteriorly on each side toward the hemispheres.

4. Ventral fissure; a median longitudinal groove along the ventral side of the medulla and spinal cord.

Draw the ventral surface of the brain and spinal cord.

e. *Peripheral Nerves:* a. Spinal Trunks. (1) *Sciatic plexus:* A number of large nerves, on each side of the dorsal aorta connected by branches and ending posteriorly in the large sciatic nerve, while anteriorly it is formed from the 7th, 8th, and 9th spinal nerves. (2) Anterior to the sciatic plexus, three pairs of small spinal nerves, the 6th, 5th, and 4th pass obliquely outward and posteriorly along the wall of the body cavity. (3) Branchial nerve; formed from the union of the 2nd, and 3rd spinal nerves; it goes to the arm.

b. Raise the dorsal aorta and notice the two slender longitudinal *sympathetic trunks* passing dorsal to it, one on each side. (1) Each trunk has numerous enlargements or ganglia giving off fine nerves. (2) Large lateral trunks connect these ganglia with the spinal nerves. (3) *Periganglionic glands;* white masses of unknown function, surrounding the spinal nerves where they issue from the spaces between the transverse processes of the vertebrae.

IV. Museum Specimens.

Observe in the Museum, south wing, numerous preparations of

organ systems of different vertebrates, and compare them with corresponding organ systems of the frog.

B. THE SKELETON.

In connection with the dried prepared skeleton, study the fresh skeleton, boiled for a *few* minutes after removing the skin and viscera.

I. General Arrangement of Parts of the Skeleton.

a. The main axis consists of the *vertebral* column continued anteriorly as the central part (brain case) of the skull.

b. Connected with the main axis are the supporting parts of the appendages, and the lateral parts of the skull.

1. The anterior appendages consist of a free limb (containing a *humerus, radio-ulna, carpus and digits,* supported by a *shoulder girdle* or *pectoral arch.*

2. The posterior appendages consist of a free limb (containing a *femur, tibio-fibula, tarsus* and *digits*) connected with the spinal column by the pelvic girdle.

II. Vertebral Column.

Made up of nine separate vertebrae and of a posterior *Urostyle.*

a. Remove the third vertebra, and note:

1. Solid flattened ventral part (*centrum*), with anterior concave, and posterior convex surfaces.

2. Neural arch; arising dorsally from the centrum and enclosing a space which forms with that of the other vertebrae, the *neural canal.*

3. Processes of the vertebrae; on each side, one *transverse process,* four *articular processes,*—one on each side of arch anteriorly (*pre-zygapophysis*), one on each side of arch posteriorly (*post-zygapophysis*),—a median *spinous process* arising from dorsal surface of the arch.

b. The first vertebra (*atlas*), articulates anteriorly by two surfaces with the skull.

The eighth vertebra (*sacrum*) has a large transverse process joining the pelvic arch and a centrum convex anteriorly and with two tubercles posteriorly to articulate with the—

c. Urostyle, a single rod-like bone continued posteriorly as a cartilage and with a dorsal ridge anteriorly, inside which is a small neural canal opening laterally by two small foramina, one on each side.

Draw the third vertebra to show the points mentioned

III. The Anterior Appendages.

a. The shoulder girdle or pectoral arch on each side is closely united to the sternum to form an incomplete ring of bones to which the free limbs are attached.

b. The paired lateral parts of the shoulder girdle are: (1) *Scapula;* large ossified plate extending dorsally from the articulation of the free limb. (2) *Supra-scapula;* a very large cartilage continued dorsally from the scapula. (3) *Coracoid;* a flat elongated bone extending ventrally and inward from the articulation of the limb towards its fellow from which it is separated by an *epicoracoid cartilage.* (4) *Procoracoid;* a cartilaginous band anterior to the coracoid and parallel to it, and more or less concealed by an ossified *clavicle* along its anterior edge.

c. The median unpaired parts are: (1) *Sternum;* a short flat bone directly posterior to the epicoracoid cartilage. (2) *Xiphisternum;* a broad bilobed cartilage extending posteriorly from the sternum. (3) *Omosternum;* a small cartilage anterior to the epicoracoid cartilages, and separated from them by the sternum.

d. Skeleton of the free-limb: (1) *Humerus;* the single bone of the brachium. (2) *Radio-ulna;* a bone representing two fused bones (*radius* and *ulna*). When the arm is extended at right angles to the body with palm ventral, the radial side is anterior, the ulnar side posterior. (3) *Carpus;* making a complex joint between forearm and digits of hand and composed of six small nodules of bone or cartilage: First, proximally two (*radiale, ulnare*), side by side, articulating respectively with radius and ulna; second, distally three (*carpalia*), of which the external is much the largest and articulates with digits 4, 3, 2, while the two small inner ones articulate with digit 1, and rudimentary digit 1^1; third, a bone (*centrale*) on the inner side of the wrist between the proximal and distal rows. (4) *Digits:* each of the four digits contains a long *metacarpal* and three long *phalanges,* except the innermost finger which has but two phalanges. A single metacarpal bone on the inner (*radial*) and palmar side represents an additional radial digit, or support for the cushion of the thumb.

Draw and label the pectoral girdle and one anterior appendage.

IV. Posterior Appendages.

a. The hip-girdle or pelvic arch on each side is firmly united with its fellow and joined to the sacrum. (1) At the *acetabulum* or articular facet for the femur, three radiating fissures mark out the division into three parts: *ilium* anteriorly articulating with the sacrum;

ischium posteriorly united with its fellow into one mass; *pubis* or part wedged in between the ilium and ischium and united with its fellow on the ventral line.

b. Bones of the free-limb. (1) In the thigh one long bone, the *femur*. (2) In the crus or leg, the *tibio-fibula* representing a fused *tibia* and *fibula*. When the leg is extended as the arm was, the anterior side is the *tibial* side. (3) *Tarsus*: this is made up chiefly of two long proximal bones fused at the ends and representing a *tibiale* (or *astragalus*) and a *fibulare* (or *calcaneum*). Between these and the digits are three small distal *tarsalia,* the inner or anterior one articulates with digit 1, the flat larger outer one with 2 and 3 and (as a cartilaginous continuation) with 4 and 5.

c. *Digits*. There are five, each with a *metatarsal* and three *phalanges,* except the first or tibial digit which has two phalanges. (1) The hard cushion on the inner side of the sole is supported by what seems an additional inner or tibial digit, represented by three short bones articulating with the same inner tarsalium that digit 1 does.

Draw and label the bones of the pelvic girdle and leg.

V. The Skull.

a. In the dry skull note the following parts and draw the whole on a large scale, as seen from above. (1) The *cranium* or brain-case. (2) The *sense-capsules,* auditory and olfactory. (3) The *facial bones* separated more or less widely from the cranium. (4) The *foramen magnum,* or passage from the neural canal into the cranium. (5) The convex *condyle* on each side of the foramen magnum corresponding to the two parts of the atlas. These are upon the *exoccipitals*. (6) *Pro-otics* or bones anterior to the exoccipitals, forming on each side the roof and anterior wall of the auditory capsule. (7) The *squamosal,* a hammer-shaped bone, running from the pro-otic downward and posteriorly towards the articulation of the lower jaws. (8) The *fronto-parietals:* two long parallel bones in the roof of the cranium with a median suture between them. (9) *Nasals*: immediately anterior to the latter and triangular in outline. (10) *Pre-maxillae*: between the nasals and the gape anteriorly. (11) *Maxillae*: extending from the latter bone on each side along nearly all the upper edge of the gape. (12) *Quadrato-jugal:* a delicate bone forming the boundary of the gape between the maxilla and squamosal on each side.

b. On the ventral surface note and draw the following:—(1) *Parasphenoid:* a dagger-shaped bone, running along the floor of the cranium or roof of the mouth. (2) *Sphenethmoid* or girdle bone:

a large bone enclosing the anterior third of the brain case. (3) *Palatines* slender bones ventral to the nasals, (on the roof of the mouth), passing outward from near the anterior end of the parasphenoid to the maxillae. (4) *Vomers* two irregular bones anterior to the last and bearing teeth (as do the premaxillae and maxillae. (5) *Pterygoids:* a tri-radial bone on each side attached to anterior wall of auditory capsule, to the squamosal and to the inner side of the maxillae. (6) The *Mandible* or lower jaw made up of a right and left half or ramus, each of which has a main piece (*angulo-splenoid*) extending from the auditory region and covered anteriorly by a scale-like external bone (*dentary*) and also a short nodular piece (*mento-Meckelian*) at its anterior tip where it joins its fellow ramus in the median line.

c. (1) In a slightly boiled or fresh skull remove carefully the *membrane or investing bones* with forceps or scalpel and draw the *chondro-cranium* that is thus laid bare, together with the following bones that remain *as ossified parts of the cartilaginous skull*: First, exoccipital; second, pro-otics; third, sphenethmoid; fourth, quadrate; fifth, mento-meckelian. (2) This cartilaginous skull forms a case for the brain, olfactory and auditory organs; on each side a long sub-ocular arch or *palato-quadrate cartilage* standing out away from the cranium is produced as a *suspensorium* at its posterior end for the articulation of the mandible; the latter is *Meckel's cartilage,* ossified at its anterior tip as the mento-Meckelian bone. (3) Note the *fontanelles* or imperfect parts of the roof of the cranium; the *foramina* for passage of nerves from the brain through the chondrocranium; the partly ossified *columella auris* extending from the tympanum to an opening, *fenestra ovalis,* in the auditory capsule.

d. *The hyoid*: this consists of a broad flat body of cartilage in the floor of the mouth, and of four pairs of processes. (1) The *anterior cornua* arising from the anterior edge of the body on each side as long slender cartilaginous rods attached near the fenestra ovalis. (2) Posterior *cornua* or *thyro-hyals:* a short thick bony piece joined on each side to the posterior edge of the body near the middle line. (3) On each lateral edge anteriorly and posteriorly (or at the angles of the body), a short rounded cartilaginous process.

Draw the hyoid.

VI. Museum Specimens.

Observe in the Museum, central section, numerous skeletons of vertebrates, and compare the bones of the appendages with those you have studied in the frog.

C. HISTOLOGY.

I. Epithelium.

a. *Columnar epithelium*: Gently scrape the inner surface of a frog's intestine that has been preserved in Müller's fluid. The fragments removed, under a high power, are seen to be composed of elongated cells each with a nucleus and having one end more pointed than the other.

b. *Ciliated epithelium*: Cut off a bit of the mucous membrane from the tongue or roof of the mouth of a freshly killed frog, mount in physiological salt solution and examine under a high power. Note the appearance on the edge due to the cilia, as the cilia become less active individual ones can be distinguished. Scrape off some of the epithelium and examine under a high power in physiological salt solution; note the shape of the individual cells and the group of cilia at one end. *Draw both kinds of epithelium.*

II. Muscle.

a. Tease out a bit of injected frog's muscle preserved in alcohol. (1) It is composed of elongated fibres, some of which may be split up somewhat into fibrillae. (2) Numerous blood capillaries are found amongst the fibres.

b. Examine with a high power: (1) Each fibre shows alternate darker and lighter bands. (2) A delicate sarcolemma or structureless membrane envelops each fibre and can be easily seen at places that are broken or twisted.

c. Tease out fresh muscle in salt solution and examine with high power to note the above points; treat with acetic acid and observe the oval nuclei in the fibre. *Draw.*

III. Nerve.

a. Nerve fibres: Tease out a bit of fresh nerve in salt solution and examine with a high power. Note: (1) Well defined fibres, each with a double contour, together with white fibrous tissue make up the mass of the nerve. (2) Each fibre has a highly refractive border (medullary sheath) and a central homogeneous *axis cylinder,* well seen in torn specimens where also the very delicate, outermost membrane (*primitive sheath*), may be some times made out.

b. Ganglion cells: Examine prepared specimens of ganglion cells that have been stained to make out the structure of the cells. *Draw a nerve fibre and a ganglion cell.*

IV. Cartilage.

Dissect out the tip of the delicate xiphisternal cartilage of a fresh frog, or slice a bit of the cartilage from the head of the femur with a razor; mount in salt solution and study under the high power. Note: (1) Large rounded cartilage cells scattered through a nearly invisible and structureless matrix which forms a refractive halo about each cell. (2) A distinct nucleus (or two) in each cell. (3) After some time the cells contract and thus a space is formed between the cell and the matrix. *Draw.*

V. Bone.

Examine with low power a section of bone (mammalian bone). Observe: (1) *Haversian canals,* rounded spaces often filled with air or dirt and then appearing black. (2) *Lamellae,* concentric layers about each haversian canal. (3) *Lacunae,* oval black spaces between the lamellae. (4) *Canaliculi,* minute dark lines radiating from the lacunae. (5) Other lamellae are to be found not arranged about canals, but filling in spaces not occupied by such systems. *Draw.*

VI. Connective Tissue.

a. White fibrous tissue: (1) Tease out a bit of fresh tendon in water; with a high power it is seen to be made up of fine wavy fibres in bundles; each fibre has faint outlines and does not branch. (2) Treat with acetic acid; most of the fibres become invisible, but a few (yellow elastic fibres) and some elongated granular connective tissue cells remain visible.

b. Yellow elastic fibres: Tease out in acetic acid some of the tissue immediately under the frog's skin. Under a high power note: Branched fibres with well defined outlines; these may not be found until several specimens have been examined. *Draw both white and yellow fibres.*

Part II.

ECOLOGY.

The following directions for laboratory work are general in character since they are intended to apply to various specimens from the Museum and Vivarium. Several specimens, illustrating different kinds of adaptations, etc., will be assigned, one after another, to each member of the class. Keep laboratory records for each specimen separate and distinct; first record the scientific name of each specimen, and then the results of observations on the topics proposed.

A. RELATIONS TO INORGANIC ENVIRONMENT.

I. Habitat.

Is the specimen a marine (Halobios), fresh-water (Limnobios) or terrestrial (Geobios) form? What are the evidences upon which your conclusion is based?

1. If aquatic, is it a bottom form (Benthos) or a top form (Plankton)? Give evidence for your conclusion.

2. If terrestrial, is it fitted for life in arid or swampy regions, or for subterranean, arboreal, or aerial life? Give reasons for your answer.

3. Are there any evidences that this animal or its ancestors have ever changed habitat? If so, what are they?

4. Draw the specimen, devoting particular attention to those adaptations which have relation to the habitat.

II. Climate.

1. Temperature. Does the animal show any particular adaptations to heat or cold? In what condition does it pass the winter? The summer?

2. Moisture. Does it show adaptations for the prevention of the loss of moisture, or to protect it against too great moisture?

3. Winds. What adaptation, if any, does it show to winds?

4. Light. Is it a form which seeks or avoids strong light, and what adaptations does it show in this connection?

III. Movement.

1. Is the animal free-moving or sedentary? (a) If free moving is it passively carried by winds or currents, or does it move actively? Do the organs of locomotion indicate that it is fitted for swimming, walking, running, creeping, leaping, burrowing, or flying? Draw one or more of the locomotor organs. (b) If sedentary is it free or attached? Show by drawings the means of attachment. Are there any rudiments of locomotor organs? Are sedentary animals descended from free-moving ones, or *vice versa?*

2. Does this species undergo periodical migrations? If so describe them.

3. In the specimen assigned you by what means is the dispersal of the species secured, and what are the barriers to such dispersal?

IV. Geographical Distribution.

1. To what Zoogeographical Region of the earth is the animal native?

B. RELATIONS TO ORGANIC ENVIRONMENT.

I. Food.

Animals are monophagous or polyphagous, depending upon whether they live upon a single kind of food or on several kinds; they are carnivorous, herbivorous, or omnivorous depending upon whether they eat flesh, plants, or both.

1. Correlation of Food and Structures. Determine by means of the organs of prehension, the teeth, or the character of the mouth parts of the speciment assigned you what is the nature of its food. Draw these characteristic structures.

2. Correlation of Food and Habits. Point out, if possible, the correlation between the food and the habits of the animal you are studying.

3. Storage of Food. Is this animal able to store up food in any form? If so describe the process.

II. Means of Defense and Offense.

1. Active. Is the animal you are studying able to defend itself actively or not? If so, draw and describe some of the organs used for this purpose.

2. Passive. If it defends itself passively describe the methods and structures by which this is done.

III. Interrelations Between Different Species.

If the species which you are studying is always associated with some other species, in which of the following groups does it belong?

1. Commensalism. The commensal alone benefits, but the host is not injured. In the case in hand which is the commensal and which the host? Is the commensal permanently fixed to the host, or free to separate on occasions?

2. Symbiosis. The symbionts derive mutual benefit from association. These also may be free or fixed.

3. Parasitism. The parasite benefits at the expense of the host. Is the parasite an endoparasite or an ectoparasite? Is it temporary or constant?

IV. Coöperation Between Individuals of the Same Species.

Associations of individuals of the same species fall under one or another of the following heads:—

a. Colonial forms without division of labor.

b. Colonial forms with division of labor.

c. Association of separate individuals without division of labor.

d. Association of separate individuals with division of labor.

(1). Does the form which you are studying belong in any of these groups? What advantage, if any, is derived from association without division of labor? If physiological division of labor is present is it associated with structural diversity? If so, draw each of the types present, and determine their relations to one another.

(2). Study a colony of bees, or of ants, and draw figures of the males, females and workers. Observe the varied activities of the members of the colony. If other members (castes, slaves) are present in the ant colony make a study of them also. Note the way in which the food is stored and the young are cared for. Study a section through honey comb, and if possible, observe the method in which it is formed. Observe and draw to scale worker cells, drone cells and queen cells, and if possible observe the kinds and relative quantities of food which are fed to the larval workers and queens.

V. Sexual Reproduction.

1. Sex. In many plants and lower animals the sexes are united in the same individual (Hermaphroditism); in higher animals the sexes are generally separate (Gonochorism). To which class does your specimen belong?

2. Primary and Secondary Sexual Characters. The ovaries and testes are primary sexual characters; all other sexual characters, which are dependent for their development upon the primary ones, are secondary sexual characters. Draw and compare the secondary characters which distinguish male and female, and, if possible, determine the significance of each.

3. Semination. Is semination internal or external? Draw the structures of the male and female which serve to bring the spermatozoa to the ova.

4. Types of Development. Where does the development of the embryo occur? Is the animal oviparous or viviparous? Do the embryos obtain their food by their own activities (larval developments), or from the mother (foetal development)?

5. Care of Eggs and Young. In the species you are studying are the eggs and young cared for? If so, how?

Part III.

GENETICS.

A. ONTOGENY. Development of the Individual.

I. Asexual Reproduction occurs by Fission, Budding, Segmentation, and has been studied in the Protozoa, Hydra, Taenia, etc. If time permits study in detail prepared slides showing the process of fission in Stenostoma.

II. Sexual Reproduction.

a. Monogony; sexual reproduction with only one parent.

1. Parthenogenesis (virgin reproduction). Observe and draw water fleas (Daphnia) containing broods of young produced from unfertilized eggs. The same phenomenon may be seen in plant lice (Aphides.)

2. Paedogenesis (infant reproduction). Observe and draw stages in the development of unfertilized eggs in the larvae (sporocysts, rediae) of the trematode worm, Diplodiscus.

b. Amphigony; sexual reproduction with two parents.

1. Oogenesis and Spermatogensis. Study prepared sections of; (1) Ovitestis (hermaphrodite gland) of the snail, Planorbis, and draw a portion of the section to show the ova, the spermatozoa, and the method of development of each. (2) Ovary of the frog, showing eggs of very different sizes. Note the enormous size of the nucleus (germinal vesicle). It is filled with nuclear sap, in which are scattered nucleoli and fine threads of chromatin. Note the distribution of the yolk and pigment in the egg. Draw. (3) Testis of frog, showing mature spermatozoa, their heads attached in bundles to nurse cells and their tails extending into the lumen of the seminiferous tubule. Around the walls of the tubule are seen the following stages in the formation of spermatozoa: (a) Spermatogonia, cells with clear nuclei, at periphery, (b) Spermatocytes I, large cells with chromatin in clumps, (c) Spermatocytes II, smaller cells with densely staining nuclei, (e) Spermatozoa, with progressively elongating nucleus and cell body.

2. Maturation and Fertilization. The last two cell divisions in the oogenesis and spermatogenesis are known as the "maturation divisions" and lead to the reduction of the chromosomes in the mature egg and sperm to half the usual number. When the egg is fertilized the normal number is again restored.

Carefully study the maturation and fertilization of the egg of Ascaris megalocephala, with especial reference to the chromosomes. Observe that in the maturation of the egg (also of the sperm) the number of chromosomes is reduced to two, and in the union of the egg and sperm the number is increased to four, the normal number. Draw eggs showing all of these points.

3. Cleavage. Observe that in the cleavage of the egg of Ascaris each chromosome is split longitudinally, so that each daughter nucleus receives two chromosomes from the egg and two from the sperm. Draw.

4. Development of Frog. Study entire frogs' eggs in various stages of cleavage.

Early Cleavage Stages. Examine eggs divided into two, four, eight and sixteen cells. Note in the two-cell stage that a grayish or slate colored area, crescentric in form, is present on one side of the egg, and that it is, as a rule, bisected by the first plane of cleavage. Note in the four-cell stage the relative distribution of the pigment on the anterior and posterior sides of the egg. Note in the eight-cell stage the relative sizes of the upper and lower cells, also the distribution of the pigment in the cells, and the location of the grey crescent. Note in the sixteen-cell stage the position of the fourth planes of cleavage in the upper and lower cells. Examine a section of one of the early cleavage stages; note the nuclei surrounded by pigment.

Later Cleavage Stages. Examine two of the later cleavage stages (Blastula stages). Note the comparative sizes of the cells in the upper and in the lower hemispheres of the embryo. Examine a section of a late cleavage stage. Note the large cleavage cavity; the thinness of the roof, and especially the character of the superficial cells at the level of the floor of the segmentation cavity. Out of these cells all of the main parts of the body develop.

Gastrula Stages. Study surface views of three gastrula stages showing: (1) The beginning of the dorsal lip of the blastopore, (2) the back-growth of the dorsal lip, and the appearance of the lateral lips, and (3) the formation of the ventral lip. At this time the blastopore is circular in outline and the yolk plug fills up its

opening. Study a longitudinal section of a gastrula stage. Compare with the section of the blastula and note all differences. Observe especially:

(1). The lifting up of the floor of the segmentation cavity.

(2). The slit-like archenteron, opening behind the dorsal lip of the blastopore.

(3). The condition of the cells in the dorsal lip itself. Draw to large scale showing ectoderm, mesoderm and endoderm.

Neural Plate. Study sections of three stages in the formation of the neural plate: (1) Stage with the neural plate widely open, (2) Sides of the neural plate rolling in, (3) Neural plate completely closed to form the neutral tube (brain and spinal cord).

Tadpole. In cross sections of a young tadpole study and draw:

(1). Origin of eye-vesicle from the fore brain, and development of the eye.

(2). Cross section through the gill region, with the heart beneath.

(3). Cross section through the ear vesicles and hind brain.

(4). Cross section through the middle of the embryo to show neural tube and crest, notochord, aorta, pronephros, somites and gut.

5. Museum Specimens. Observe in the Museum, south wing, numerous preparations illustrating the development of vertebrates.

III. Combinations of Sexual and Asexual Reproduction.

1. Metagenesis. Alternation of asexual reproduction with sexual, as in hydromedusae.

2. Heterogeny. Alternation of monogonic reproduction with amphigonic, as in fluke worms.

IV. Heredity. Germinal likeness or variation as contrasted with environmental.

1. Mendelian (alternative) inheritance. Study and draw Museum exhibits illustrating this kind of inheritance.

2. Give Mendelian formulas and ratios to the third filial generation (F^3) for the offspring of (a) two homozygous parents, (b) two heterozygous parents, and (c) one homozygous and one heterozygous parent. Explain sex as a mendelian character and show by formulas and ratios in which of these three groups it belongs.

3. Describe any cases of inheritance, known to you, which seem to be non-Mendelian.

4. All members of the class are invited, but not required, to fill out a Family Record blank, giving details of their own heredity for the use of the Committee on Eugenics.

B. PHYLOGENY. Development of Races, Species and larger subdivisions.

I. Varieties and Species.

1. Varieties. In a large series of individuals of the same species pick out and draw individuals which represent the mean and the extremes of variation.

2. Species. In a genus containing a large number of species, pick out and draw species which represent the mean and the extremes of variation.

II. Homologies.

a. Comparative Anatomy.

1. Draw and label corresponding parts of the limb skeletons of three vertebrates, having different modes of locomotion.

2. Draw and label corresponding teeth of three vertebrates, which eat different kinds of food.

3. Draw and label corresponding parts in the appendages of a lobster, or crayfish, and a crab.

4. Draw and label corresponding parts of the skeleton of a starfish and a sea-urchin.

How are such likenesses (homologies) to be explained?

b. Comparative Embryology.

1. Study and draw the adult and larva of an ascidian, and of a barnacle, and show how embryology throws light on relationships.

2. Compare the branchial clefts in an embryo chick and shark, and indicate the phylogenetic significance of this resemblance.

c. Paleontology.

2. Study in the Museum the paleontological history of some family of animals, and point out its phylogenetic significance.

III. Experiment.

With the aid of books which will be assigned you, describe the principal races of some one domestic animal or cultivated plant and compare them with the original wild stock.

IV. Factors of Evolution.

Explain the origin of the peculiar structures of the specimen assigned you according to the following theories: (1) Lamarckism. (2) Darwinism. (3) Orthogenesis. (4) Mutation.